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CYTOTOXICITY EVALUATION OF ALGAL EXTRACTS USING THREE SEP-ART ARTEMIA (INVE) GROUPS

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ABSTRACT

The study was conducted to determine the cytotoxic effects of different algal extracts on Artemia sizes and to assess how organism size influences sensitivity to algal bioactive compounds. The cytotoxicity of chloroform, acetone, methanol, hexane and ethanol extracts of five algal species (*Chlorella sp*., *Sargassum sp*., *Spirulina sp*., *Ulva sp*. and *Schizochytrium sp.*) on three Artemia groups (Sep-Art 430µm, Sep-Art 480µm and Sep-Art- 500µm) were investigated. Five concentrations (5000 µg/mL, 2500 µg/mL, 1000 µg/mL, 500 µg/mL and 100 µg/mL) of algal extracts were used in the study. In addition, the effects of different solvents on the extraction yields of the tested algae were revealed within the scope of the study. The experiment was conducted under a Completely Randomized Design (CRD) with three replicates for each treatment. When the LC_{50} values obtained from the study were classified, no highly toxic values (≤100 µg/mL) were observed. The results indicated that LC_{50} values tended to decrease as Artemia size increased (Sep-Art 430 µm≤Sep-Art 480 µm≤Sep-Art 500 µm). The Sep-Art 500 µm group exhibited higher cytotoxic activity and mortality compared to the smaller size groups ($p \leq 0.05$). Extraction yields varied significantly among solvents and algal species ($p \leq 0.05$), with the highest yield observed in *Chlorella sp.* (methanol extract, 19.33 ± 0.003%) and the lowest in *Ulva sp.* (hexane extract, 1.62 ± 0.116%). In conclusion, no highly toxic effects (≤100 µg/mL LC_{50}) were observed in any of the three Artemia groups at different algal extract concentrations. The Sep-Art 430 µm group exhibited better stress tolerance than the Sep-Art 480 µm and Sep-Art 500 µm groups. The findings of this study, which evaluated the lethality test performances of three Sep-Artemia (INVE) groups, indicate that the Sep-Art 430 µm and Sep-Art 480 µm groups are suitable for use in Brine Shrimp Lethality Assay (BSLA) tests.

Key words: Algae, Artemia, cytotoxicity, extraction yields, lethality

INTRODUCTION

Algae are among the most important primary producers in marine ecosystems, contributing substantially to global biomass production (Bhadury and Wright, 2004). Many marine organisms, including algae, produce toxic compounds as defense mechanisms against predators and environmental stressors. Therefore, toxicity testing is essential to evaluate the lethality of both biological and non-biological materials (Hood, 2009; Hamrun *et al.,* 2020). Among marine organisms, macroalgae are particularly noteworthy for their diverse secondary metabolites, which exhibit remarkable cytotoxic and bioactive potential (Manilal *et al.,* 2009; Synytsya *et al.,* 2010; Gotteland *et al.,* 2020; Maray *et al.,* 2023; Yadav *et al.,* 2024; Tubril *et al.,* 2024).

Especially, microalgae are capable of producing a wide variety of bioactive compounds as a result of their adaptation to different environmental conditions to survive with stress. The bioactive compounds obtained from microalgae have been reported to exhibit significant biological activities including antitumor, antiviral and antioxidant properties (Osman and Omar 2019).

Macroalgae have a high diversity of species known as 2000 Ochrophyta (Brown), 6500 Rhodophyta (Red) and 1500 Chlorophyta (Green) (Gutiérrez-Rodríguez *et al.,* 2018; Nazarudin *et al.,* 2021; Babich *et al.,* 2022). Macroalgae are regarded as valuable sources of antitumor compounds and a wide range of bioactive molecules with potential antioxidant, immunostimulant, anticancer, anti-inflammatory, neurodegenerative diseases, and antibacterial activities (Boujaber *et al.,* 2013; Aramanadka *et al.,* 2018; Alves *et al.,* 2018; Barbalace *et al.,* 2019). In particular, brown algae are a rich source of diverse bioactive compounds (Cardozo *et al.,* 2007; Zubia *et al.,* 2009; Biris-Dorhoi *et al.,* 2020). *Sargassum tenerrimum*, one of the brown macroalgae that has attracted attention in pharmaceutical research, promises hope for the future (Kumar *et al.,* 2013).

Artemia is an invertebrate belonging to the Crustacea class and is widely used in aquaculture studies and toxicity tests (Osman and Omar 2019; Bhatt *et al.,* 2016). Historically, Artemia has served as a popular biological model to assess the efficacy of toxic compounds (Libralato *et al.,* 2016; Turan and Mammadov 2021). Toxicity testing using the Brine Shrimp Lethality Assay (BSLA) is a widely accepted and reliable method for evaluating the bioactivity of algal extracts and natural products (Banti and Hadjikakou, 2021; Syamsurizal *et al.,* 2023). The use of Artemia offers several advantages, including simplicity, rapid results, low cost, reproducibility, and the absence of ethical concerns (Hamrun *et al.,* 2020). Moreover, Artemia-based assays are suitable for determining dose–response relationships (Chan *et al.,* 2021).

The physiological characteristics and toxin sensitivity of Artemia may vary with size, influencing the accuracy of lethality tests. Understanding these differences can enhance the reliability of BSLA and support the selection of optimal test sizes for toxicological, pharmacological, and aquaculture applications. Previous BSLA studies have primarily used Artemia larger than 500 µm (Sorgeloos *et al.,* 1978; Meyer *et al.,* 1982; McLaughlin *et al.,* 1998; Carballo *et al.,* 2002; Sarah *et al.,* 2017; Kamanja *et al.,* 2018; Banti *et al.,* 2021).

This study aimed to evaluate the cytotoxic effects of various algal extracts (*Chlorella sp.*, *Sargassum sp.*, *Spirulina sp.*, *Ulva sp.*, and *Schizochytrium sp.*) prepared with different solvents (chloroform, acetone, methanol, hexane, and ethanol) on Artemia of different sizes (Sep-Art 430 µm, Sep-Art 480 µm, and Sep-Art 500 µm), and to determine how organism size influences their sensitivity to algal bioactive compounds. Furthermore, the study investigated the effects of solvent type on extraction yield.

MATERIALS AND METHODS

Preparation of algal extracts: The algal species (*Chlorella sp.*, *Sargassum sp.*, *Spirulina sp.*, *Ulva sp.*, and *Schizochytrium sp.*) used in the study were obtained from a commercial company. Briefly, algae were first extracted in Chloroform, Acetone, Methanol, Hexane and Ethanol solvents at a concentration of 12.5 g/100 mL. The extraction was performed for 24 hours at room temperature. The procedure was repeated twice using the same conditions. After the obtained extract was filtered through Whatman filter paper No. 1, the solvent was evaporated using a rotary evaporator. Extracts were kept at -20^{o}C (Krishnaraju *et al.*,2005; Turan and Mammadov, 2021).

Artemia hatching: Artemia eggs were purchased from commercial company (INVE). INVE Aquaculture's patented SEP-Art® technology provides a magnetic coating on the cysts. Artemia eggs (Sep-Art 430µm, Sep-Art 480µm and Sep-Art- 500µm) were hatched in a 1000 mL beaker containing 800 mL artificial seawater prepared with distilled water and kept under at 28°C under continuous aeration and illumination for approximately 24 h. The artificial seawater was prepared by dissolving sea salt (38 g) in 1000 ml water. After 24 h, the Artemia nauplii were collected. This widely used method ensures optimum viability and health of Artemia nauplii for bioassay applications. (Naz, 2008)

Cytotoxic activity: A completely randomized design (CRD) was used to analyse the data of this experiment. Each algal species was tested seprately against five concentrations of each extract. In this experimental, a total of 900 Artemia nauplii were used for each algal species. During the study, 4500 Artemia nauplii (900 for each of the five algal species) were used for each of the Sep-Art 430 µm, 480 µm, and 500 µm. Algal extracts were dissolved in Dimethyl sulfoxide (1% DMSO) and then tested at 5000 , 2500 , 1000 , 500 and 100 µg/mL. After hatching, 10 Artemia nauplii were placed in vials containing three replicates of each concentration extracts ranging from 100 µg/mL to 5000 µg/mL. Control group was treated without addition of algal extract to the seawater and that containing 1% DMSO (100% survival). The toxicity assay was carried out over a 24-hour period (Jeda *et al.,* 2014)

After 24 h, the number of surviving Artemia at each concentration of the algal extracts were counted and the LC_{50} values of the concentrations required to kill 50% of the Artemia were calculated by using a Probit regression analysis (Finney,1971). Mortality of Artemia nauplii was assessed based on the absence of visible movement during a defined observation period of 30 seconds. The cytotoxic effect was observed by calculating the mortality number of Artemia.

Clarkson's toxicity criterion classifies were used for LC_{50} values. According to this classification, LC_{50} value is considered non-toxic greater than 1000 µg/mL, extracts between 500 µg/mL and 1000 µg/mL are considered low toxic, extracts between 100 µg/mL and 500 µg/mL are medium toxic, and extracts $\leq$100 µg/ml are considered highly toxic (Hamidi *et al.,* 2014; Hamrun *et al.,* 2020).

Statistical analysis: The toxicity of the extracts was evaluated using the Brine Shrimp Lethality Assay (BSLA) as described by Meyer *et al.* (1982). Results were expressed as mean ± standard error (SE). Lethal concentrations (LC_{50}) were calculated using Probit regression analysis according to Finney (1971) in SPSS (IBM Corp.). Differences among groups were analyzed by one-way ANOVA followed by Duncan's multiple range test, with significance set at $p \leq 0.05$.

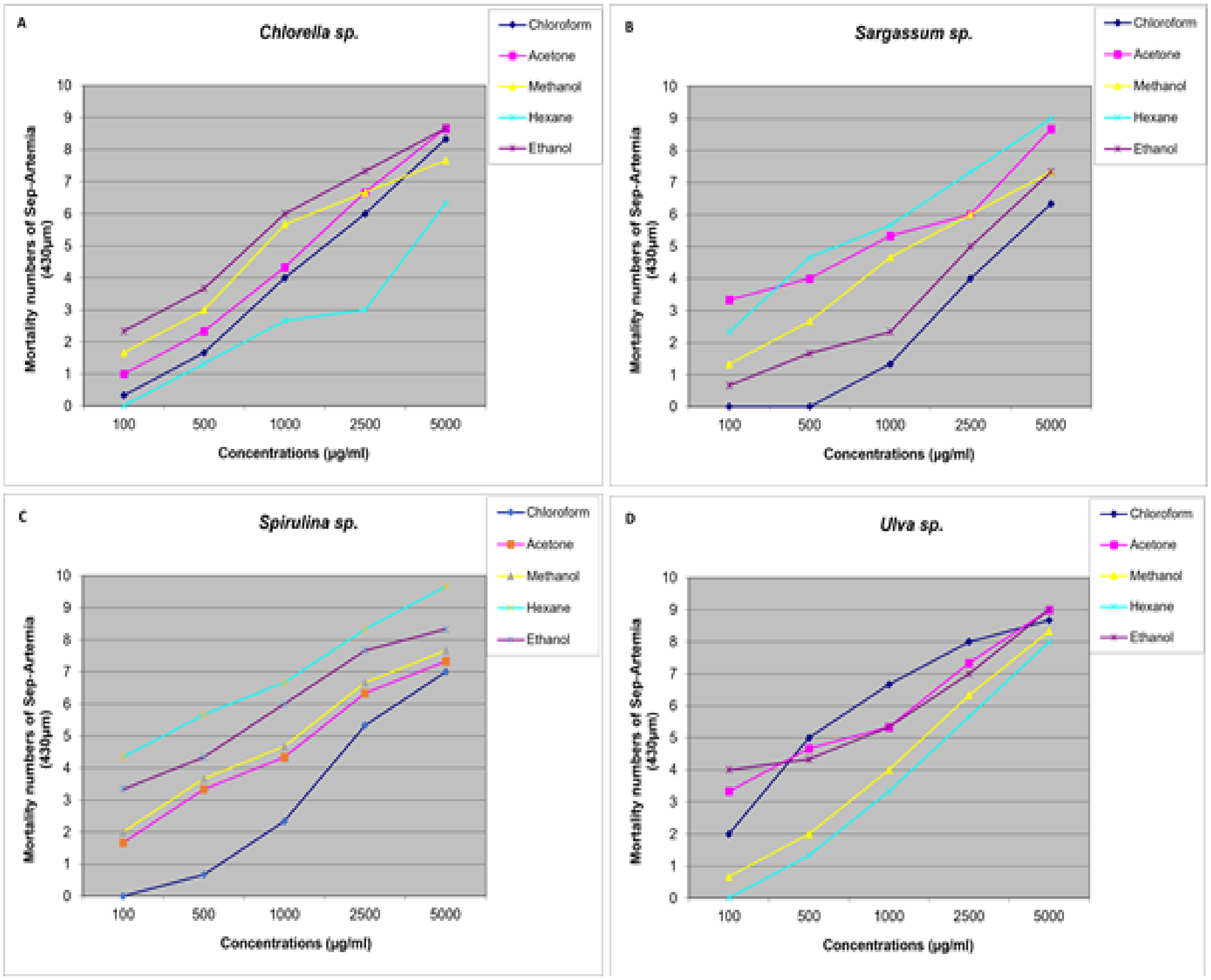
RESULTS

In current study, BSLA was carried out to investigate the cytotoxicity of the algal extracts (*Chlorella sp*., *Sargassum sp*., *Spirulina sp*., *Ulva sp*. and *Schizochytrium sp*.) obtained from different solvents (Chloroform, Acetone, Methanol, Hexane and Ethanol) on three Artemia groups (Sep-Art 430µm, Sep-Art 480µm and Sep-Art- 500µm). Artemia groups were exposed to different concentrations (5000 µg/mL, 2500 µg/mL, 1000 µg/mL, 500 µg/mL and 100 µg/mL) of algal extracts for 24 h.

LC_{50} concentrations of the algal extracts were determined using Probit regression analysis (Tables 1–3). For all Artemia sizes, most extracts were classified as low or non-toxic, while a limited number showed medium toxicity (100–500 µg/mL). LC_{50} values generally decreased with increasing Artemia size (Sep-Art 430 µm ≤Sep-Art 480 µm ≤Sep-Art 500 µm), indicating higher cytotoxicity in larger individuals.

The extraction yields of the algae extracted with Chloroform, Acetone, Methanol, Hexane and Ethanol solvents were given in Table 4. The differences observed in extraction yields were statistically significant ($p \le 0.05$). The highest extraction yield was obtained for *Chlorella sp.* in methanol (19.33 ± 0.003%), whereas the lowest yield was observed for *Ulva sp.* in hexane (1.62 ± 0.116%).. The highest extraction yields of tested algae were determined in methanol extracts. The lowest extraction yields were found in hexane extracts except for *Sargassum sp*.-Chloroform extract. In generaly, Schizochytrium *sp*. had higher extraction yields than those of tested other algae.

Figures 1–3 show the effects of different concentrations (100–5000 µg/mL) of *Chlorella sp.*, *Sargassum sp.*, *Spirulina sp.*, *Ulva sp.*, and *Schizochytrium sp.* extracts on the mortality of Sep-Art 430 µm, 480 µm, and 500 µm. Overall, mortality increased with Artemia size, with Sep-Art 500 µm exhibiting the highest mortality, followed by Sep-Art 480 µm and Sep-Art 430 µm after 24 hours.



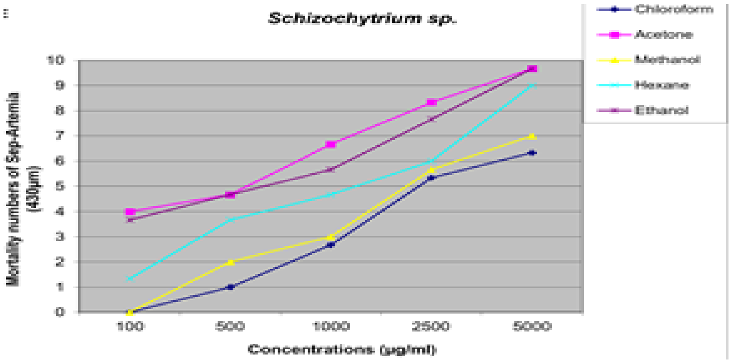


Figure 1. The effects of tested algae extracts on Sep Artemia (430µm). A. The effects of different concentrations of *Chlorella sp.* B. The effects of different concentrations of *Sargassum sp.* C. The effects of different concentrations of *Spirulina sp.* D. The effects of different concentrations of *Ulva sp.* E. The effects of different concentrations of *Schizochytrium sp.*

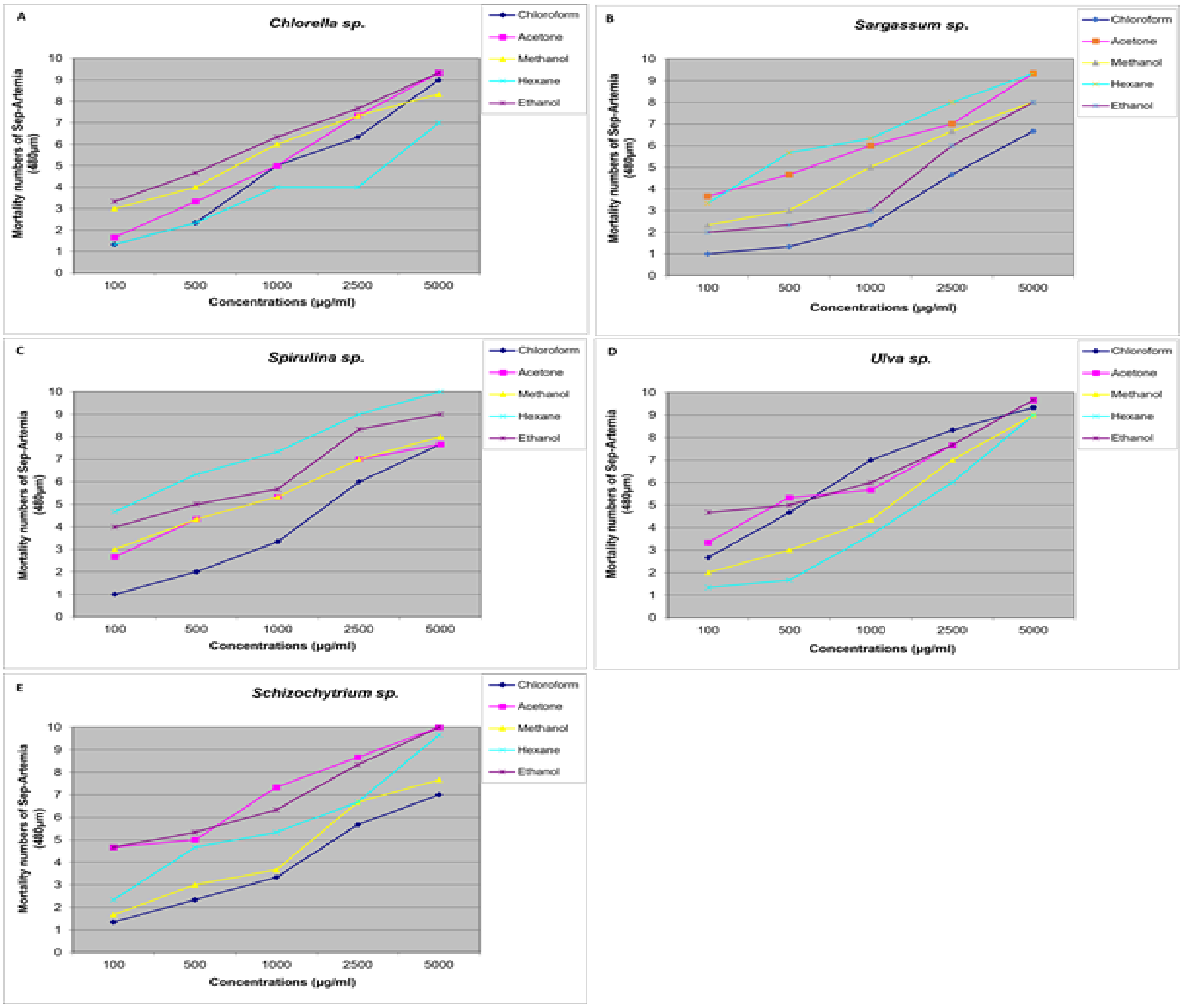


Figure 2. The effects of tested algae extracts on Sep Artemia (480µm). A. The effects of different concentrations of *Chlorella sp.* B. The effects of different concentrations of *Sargassum sp.* C. The effects of different concentrations of *Spirulina sp.* D. The effects of different concentrations of *Ulva sp.* E. The effects of different concentrations of *Schizochytrium sp.*

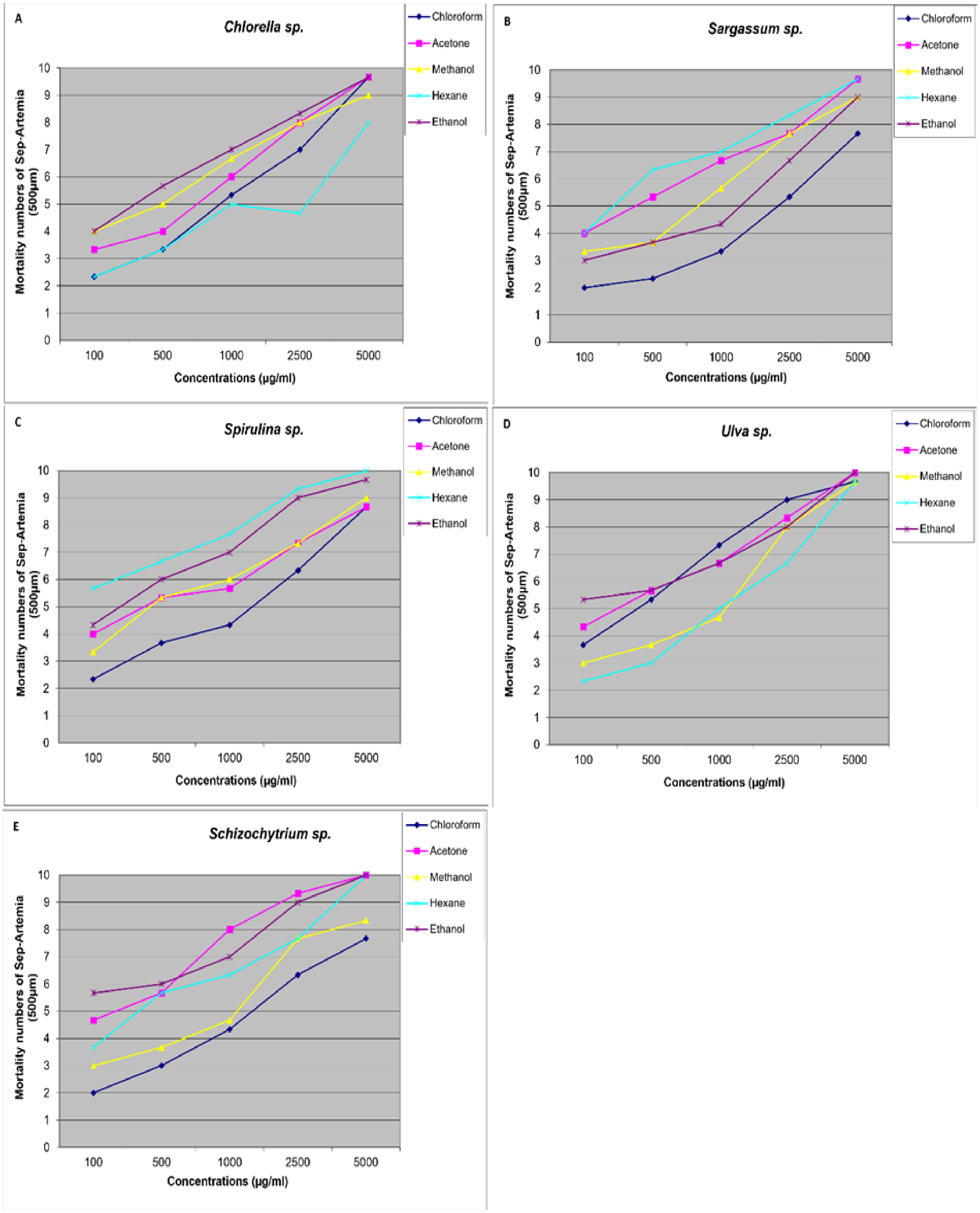


Figure 3. The effects of tested algae extracts on Sep Artemia (500µm). A. The effects of different concentrations of *Chlorella sp.* B. The effects of different concentrations of *Sargassum sp.* C. The effects of different concentrations of *Spirulina sp.* D. The effects of different concentrations of *Ulva sp.* E. The effects of different concentrations of *Schizochytrium sp.*

Table 1. LC_{50} Concentrations of Sep-Art 430µ (µg/ml).

Species	LC_{50} Concentrations of Sep-Art 430µ				
	Chloroform	**Acetone**	**Methanol**	**Hexane**	**Ethanol**
Chlorella sp.	1553.226 (849.42-3191.15) * R^2=0.985	1177.621 (577.12-2542.9) $R^2$0.959	981.032 (337.05-2822.55) R^2=0.96	3542.706 (1818.58-24780.07) R^2=0.909	635.468 (181.44-1502.99) R^2=0.957
Sargassum sp.	3367.987 (2132.51-8366.32) R^2=0.999	623.50 (42.611-2413.85) R^2=0.946	1386.977 (550.09-5171.2) R^2=0.98	559.759 (146.23-1288.13) R^2=0.964	2354.369 (1180.25-9054.66 R^2=0.936
Spirulina sp.	2483.249 (1487.77-5603.25) R^2=0.989	1230.948 (419.48-5168.51) R^2=0.988	974.511)281.63-3433.37) R^2=0.983	226.751 (10.04-575.08) R^2=0.989	452.910 (35.59-1271.10) R^2=0.946
Ulva sp.	491.712 (131.61-1059.71) R^2=0.995	464.490 (53.64-1237.44) R^2=0.992	1409.133 (727.02-3087.22) R^2=0.975	1871.456 (1092.76-3699.95) R^2=0.993	423.595 (11.02-1322.16) R^2=0.99
Schizochytrium sp.	2620.825 (1474.6-7672.04) R^2=0.974	297.832 (36.144-694.33) R^2=0.992	2087.088 (1136.52-5389.5) R^2=0.992	977.142 (407.22-2304.14) R^2=0.922	383.592 (53.87-920.1) R^2=0.978

*95% Confidence limits for LC_{50} Concentrations (Covariate)

Table 2. LC_{50} Concentrations of Sep-Art 480µ (µg/ml).

Species	LC_{50} Concentrations of Sep-Art 480µ				
	Chloroform	**Acetone**	**Methanol**	**Hexane**	**Ethanol**
Chlorella sp.	1051.651 (486.68-2317.03) * R^2=0.911	779.220 (333.12-1603.43) R^2=0.929	540.001 (74.09-1499.41) R^2=0.948	2372.313 (899.54-52832.33) R^2=0.924	389.961 (58.26-925.33) R^2=0.908
Sargassum sp.	2970.836 (1327.71-27679.21) R^2=0.996	392.636 (28.77-1048.27) R^2=0.958	924.807 (269.99-3045.80) R^2=0.923	324.360 (37.54-768.90) R^2=0.947	1500.453 (597.44-6795.09) R^2=0.995
Spirulina sp.	1683.3 (804.26-5152.32) R^2=0.95	675.779 (77.43-2429.37) R^2=0.988	602.639 (51.11-2074.44) R^2=0.965	171.322 (9.90-415.68) R^2=0.931	319.626 (15.93-843.11) R^2=0.977
Ulva sp.	402.706 (106.45-839.89) R^2=0.973	376.354 (58.01-878.09) R^2=0.957	890.073 (355.88-2108.45) R^2=0.888	1343.434 (666.40-3185.24) R^2=0.99	255.241 (3.18-715.49) R^2=0.972
Schizochytrium sp.	1905.663 (789.71-11682.12) R^2=0.951	214.304 (16.91-509) R^2=0.956	1260.516 (489.13-4354.25) R^2=0.937	588.303 (173.94-1324.56) R^2=0.921	227.730 (11.04-571.2) R^2=0.999

*95% Confidence limits for LC_{50} Concentrations (Covariate)

Table 3. LC$_{50}$ Concentrations of Sep-Art 500µ (µg/ml)

Species	LC$_{50}$ Concentrations of Sep-Art 500µ				
	Chloroform	**Acetone**	**Methanol**	**Hexane**	**Ethanol**
Chlorella sp.	674.891 (248.57-1463.36) * R^2=0.969	429.743 (101.33-949.1) R^2=0.992	285.447 (9.58-763.45) R^2=0.934	1202.963 (282.79-11628.12) R^2=0.874	243.067 (19.37-583.67) R^2=0.903
Sargassum sp.	1713.947 (637.7-13663.41) R^2=0.999	275.504 (16.4-692.64) R^2=0.954	504.892 (93.22-1264.82) R^2=0.99	216.568 (12.37-534.5) R^2=0.913	721.307 (167.24-2157.03) R^2=0.989
Spirulina sp.	880.578 (265.1-2680.06) R^2=0.985	322.018 (0.492-1031.98) R^2=0,903	378.425 (27.14-995.35) R^2=0.934	107.767 (0.634-309.25) R^2=0.998	203.193 (13.63-491.13) R^2=0.923
Ulva sp.	265.042 (46.18-572.34) R^2=0.946	230.565 (16.95-554.6) R^2=0.929	560.610 (176.42-1232.97) R^2=0.996	755.163 (287.65-1702.22) R^2=0.964	158.833 (0.189-479.3) R^2=0.997
Schizochytrium sp.	1168.993 (388.43-4764,93) R^2=0.946	179.941 (18.81-406.9) R^2=0.98	658.096 (133.45-1898.93) R^2=0.968	300.085 (36.14-697.16) R^2=0.993	127.475 (0.423-373.47) R^2=0.998

*95% Confidence limits for LC$_{50}$ Concentrations (Covariate)

Table 4. Extraction yields obtained from different solvents of algae (%).

Species	Solvents				
	Chloroform	**Acetone**	**Methanol**	**Hexane**	**Ethanol**
Chlorella sp.	4.36±0.087^{c}	12.88±0.262^{d}	19.33±0.003^{d}	1.09±0.07^{a}	4.74±0.443^{b}
Sargassum sp.	2.27±0.024^{a}	4.35±0.073^{ab}	6.67±0.102^{a}	3.89±0.141^{c}	3.38±0.429^{a}
Spirulina sp.	8.25±0.337^{d}	4.72±0.068^{b}	15.91±0.131^{c}	3.62±0.116^{c}	11.25±0.352^{c}
Ulva sp.	2.94±0.047^{b}	4.17±0.097^{a}	9.67±0.162^{b}	1.62±0.116^{b}	5.01±0.462^{b}
Schizochytrium sp.	14.91±0.258^{e}	10.02±0.003^{c}	19.19±0.455^{d}	3.70±0.318^{c}	10.1±0.208^{c}

^{abcde}Values with differetn superscripts in a column differ signifaicntly ($p \leq 0.05$).

DISCUSSION

BSLA is an important tool for the preliminary cytotoxicity assay. Researchers showed that the highest activities of water extracts of *S. indica* and *C. racemosa* was were 64 µg/mL and 67 µg/mL, respectively. LC$_{50}$ values of hexane extracts of *S. marginatum* and *S. swartzii* were 349 µg/mL and 61 µg/mL, respectively. LC$_{50}$ values of the methanol extracts of *S. binderi* and *S. asperum* were 121 µg/mL and 415 µg/mL, respectively. LC$_{50}$ values of ethanol extracts of *S. asperum, S. indica*, *S. marginatum, C. racemosa*, *S. swartzii* and *S. binderi* were found as 443 µg/mL, 507 µg/mL, 612 µg/mL, 929 µg/mL, 928 µg/mL, 735 µg/mL, respectively (Ara *et al.,* 1999). Yudiati *et al.* (2012) determined that the LC$_{50}$ value of *Spirulina sp*. was 113.20 µg/mL. Boujaber *et al.* (2013) determined that *Cystoseira humilis* showed the highest cytotoxic activity. Rizkina *et al.* (2013) found that the LC$_{50}$ of *Spirulina platensis* extracted with methanol was 446.68 µg/mL. Zahro (2014) found that the LC$_{50}$ of *Chlorella sp*. ranged from 267 µg/mL to 415 µg/mL. Afif *et al.* (2015) determined that *Eucheuma cottoni* had high level toxicity against Artemia salina according to LC$_{50}$ value. Agustini and Kusmiati (2017) showed that the LC$_{50}$ values of exopolysaccharides and endopolysaccharides of *Porphrydium cruentum* were 513.18 µg/mL and 521.82 µg/mL, respectively. Kim and Choi (2017) demonstrated significant larvicidal activity in 2.5% methanol extracts of brown algae (*Dictyota dichotoma* and *Sargassum sagamianum*,) and green algae (*Enteromorpha linza* and *Ulva pertusa*). Osman and Omar (2019) showed that the LC$_{50}$ values of mixed microalgae ranged from 403.98 µg/mL to 595.79 µg/mL and can be used safely as feed raw materials for animals. Dini *et al*. (2019) revealed that N-hexane (134.90 µg/mL), ethyl acetate (281.84 µg/mL), acetone (338.84 µg/mL) and ethanol (295.12 µg/mL) extracts of macroalgae *H. cylindracea* were toxic according to their

LC_{50} values. Hamrun *et al.* (2020) revealed that the LC_{50} value of *Eucheuma spinosum* red algae was 58.82 µg/mL and that this alga has antitumor potential. According to Premarathna *et al.* (2020), the red macroalgae species had significantly lower LC_{50} values when compared to all other brown and green algae samples. Muhammad *et al.* (2022) showed that different extracts of fresh and dry brown algae (*Padina pavonica*) exhibited good larvicidal activity against brine shrimp. Researchers revealed that the LC_{50} in the fresh samples were higher than those in the dry samples. *Ulva lactuca* had the highest LC_{50} value of all the green seaweed samples tested in the study. Also, *Ulva lactuca* and *Padina antillarum* did not cause mortality. Sunaryo *et al.* (2024) pointed out that sulfated polysaccharides can be considered safe and may trigger further research on the pharmacological potential or biomedical applications of these polysaccharides. Tubril *et al.* (2024) determined that the LC_{50} values for the algal extract and the positive control ($K_2Cr_2O_7$) was 92.38 µg/mL and 125.28 µg/ml, respectively. Yadav *et al.* (2024) revealed that the methanolic extract of *Sargassum tenerrimum* showed a toxic effect at higher concentrations on the zebrafish model but not at lower concentrations. Ara *et al.* (1999) pointed out that cytotoxic activity may be due to the difference in polarity of the compounds. Both previous studies and our findings indicate that most algal extracts exhibit low to moderate toxicity. Cytotoxicity increased with Artemia size (Sep-Art 430 µm ≤ Sep-Art 480 µm ≤ Sep-Art 500 µm), with larger individuals showing greater sensitivity. Variations in LC_{50} values may be attributed to differences in compound polarity, algal extract types, and Artemia size, in agreement with previous reports.

Elnabris *et al.* (2013) showed that the extraction yields of *Padina pavonica*, *Enteromorpha compressa* and *Ulva lactuca* were 5.2%, 7.3% and 17%, respectively. Puspita *et al.* (2017) revealed that the extraction yields of enzyme extract and aqueous extract of dry algae material were 32.6 ± 4.9% and 26.5 ± 4.7% and respectively. Güner (2017) found that the extraction yields of green and brown macroalgae were 195 mg (0.22%-chloroform)-927 mg (1.1%-methanol) and 228 mg (0.21%-chloroform)-3902 mg (3.6%-methanol), respectively. Extraction yields obtained from innovative extraction methods such as Supercritical CO_2 Extraction (SC-CO_2), Microwave Assisted Extraction (MAE) and Pressurized Liquid Extraction (PLE) were determined as 30.20 mg/g-780 mg/g, 5–20% and 40%, respectively (Roh *et al.,* 2008; Sánchez-Camargo *et al.,* 2016; Fabrowska *et al.,* 2016; Yuan *et al.,* 2018; Otero *et al.,* 2019). Park *et al.* (2023) determined that the extraction yields of brown macroalgae were between 68.40% and 81.88%. Current study, the highest and lowest extraction yields were observed in 19.33±0.003% (*Chlorella sp.*-Methanol)- 19.19±0.455% (*Schizochytrium sp.*-Methanol) and 1.62±0.116% (*Ulva sp.*-Hexane), respectively. The highest extraction yields of tested algae were determined in methanol extracts. The lowest extraction yields were found in hexane extracts except for *Sargassum sp.*-Chloroform extract. In general, *Schizochytrium sp.* had higher extraction yields than those of tested other algae. Kuda *et al.* (2005), Hayouni *et al.* (2007) and Hashem *et al.* (2021) pointed out that the extraction yield depends on the solvent polarity and that the extraction efficiency of polar solvents is high. Overall, these results confirm that both algal species and solvent polarity are critical determinants of extraction yield, and optimizing solvent selection can maximize the recovery of bioactive compounds from algae. The higher yields observed in methanol extracts in this study further support the importance of solvent polarity in extracting bioactive components efficiently.

Evaluation of Artemia mortality across Figures 1–3 revealed that mortality increased with size (Sep-Art 430 µm ≤ Sep-Art 480 µm ≤ Sep-Art 500 µm) over the 24-hour exposure period. The Sep-Art 500 µm group exhibited the highest mortality, followed by Sep-Art 480 µm and Sep-Art 430 µm. This size-dependent response may be related to differences in energy reserves, as smaller Artemia likely possess more efficient energy management, enabling them to better cope with stress induced by exposure to algal extracts.

In conclusion, the results of this study indicate that most algal extracts exhibit low to medium cytotoxicity, with toxicity increasing with Artemia size (Sep-Art 430 µm ≤ Sep-Art 480 µm ≤ Sep-Art 500 µm). Variations in LC_{50} values are influenced by compound polarity, algal species, extract type, and Artemia size, emphasizing the importance of these factors in toxicity assessments. Methanol extracts generally provided the highest extraction yields, while hexane extracts produced the lowest, highlighting the critical role of solvent polarity in maximizing bioactive compound recovery. *Schizochytrium sp.* demonstrated relatively higher extraction efficiency among the tested algae. No highly toxic effects were observed in any of the three Artemia groups, and Sep-Art 430 µm showed better stress tolerance compared to larger groups. Overall, the Sep-Art 430 µm and Sep-Art 480 µm groups can be safely used in BSLA tests. For future studies, it is recommended to include Artemia of different sizes to expand the LC_{50} range and further investigate size-dependent cytotoxicity.

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Data Availability: Data is provided within the manuscript or supplementary information files.

Ethics approval: Since no human or animal subjects were used in this study, ethical approval was not required.

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