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# PRELIMINARY TOXICITY TEST AND PHYTOCHEMICAL SCREENING OF *SARGASSUM POLYCYSTUM* CRUDE EXTRACTS FROM MARINE MACROALGAE

Norhayati Daud[1], Nor Nafizah Mohd Noor[2], Hasimah Alimon[3] and Nursuhaidah Abdul Rashid[4]

[1,2,3,4]*Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak Darul Ridzuan, Malaysia.*

[1]*norhayati.daud@fsmt.upsi.edu.my;* [2]*nafizah@fsmt.upsi.edu.my;*
[3]*hasimah@fsmt.upsi.edu.my;* [4]*suhaidah_rashid@yahoo.com.my*

ABSTRACT

Sargassum polycystum is a species of brown algae (Phaeophyta). Some recent studies have found many uses of this algae, one of them is for medicine. In this study, crude hexane, dichloromethane and methanol extracts of Sargassum polycystum were tested for *toxicity activity and screened for its principal chemical substances.* The toxicity test was done using *Brine Shrimp Lethality (BSL) test against Artemia salina Leach.* The results of this study *showed* the $LC_{50}$ value of methanol extract was 295.12 $mgml^{-1}$, while both *hexane and* dichloromethane extracts were 275.42 $mgml^{-1}$. The BSL test exhibited no significant toxicity against A.salina. Phytochemical screening *shows S. polycystum contained non polar and polar component*s. As the conclusion, *S. polycystum contained secondary metabolites that are not toxic to A. salina and they are not a potential source for toxicity.*

**Keywords:** *Sargassum polycystum;* toxicity test; phytochemical screening; BSL(Brine Shrimp Lethality) Test.

## 1. INTRODUCTION

Marine macroalgae species have the capacity to synthesize a great variety of phytochemical compounds that are known as secondary metabolites. Research of marine macroalgae has made significant advances in recent years and has shown to produce a variety of compounds that possess biological activity of potential medicinal value (Konig, Wright, Sticher, Angerhofer, & Pezzuto, 1994; Nurul, Darah, Shaida & Nor, 2010). Secondary metabolites in marine macroalgae include phenolic compounds, carotenoids and diterpenoids are characterized by the presence of halogen compounds, alkaloids, flavonoids and tannin (Nurul, Darah, Shaida & Nor, 2010). These metabolites isolated from macroalgae have shown to exhibit bioactive effect which give interest in pharmacology and pharmacognosy study

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especially in the discovery of new chemical and pharmacological products (O'Sullivan et al., 2011).

Quite recently marine algae have been given attention due to the presence of alginate, agar-agar and carrageenan. Alginate is usually found in brown algae (Phaeophyta) or seaweed, acts as an important ingredient in cosmetic industries especially for making soap, cream, lotion and shampoo as well as in the pharmaceutical industries for manufacturing emulsifiers, stabilizers, tablets, ointments, capsules and filters (Abowei & Ezekiel, 2013). Several chemical processes also need this substance as an additive in textile and ceramic industries (Abowei & Ezekiel, 2013). A number of *Sargassum* species are utilized traditionally mainly as food and medicine. *Sargassum* sp, *Caulerpa* sp, *Euchema* sp and *Gracilaria* sp. are some of the important marine macroalgaes that had been identified as rich sources for bioactive compounds (Cabrita, Vale, & Rauter, 2010; Siregar, Sabdono, & Pringgenies, 2012). *Sargassum polycystum* is widely distributed along Port Dickson beaches of Malaysia within 20-200 meters off the seashore. This edible macroalgae has been studied and was found to show effect on diabetic problem (Mahsa, Mahdi, Yong, Hemn, Mohd, & Suhaila, 2014) and has potential as antibacterial agent (Chiao-Wei, Siew-Ling, & Ching-Lee, 2011).

The aim of the present work was to study the toxicity of *S. polycystum* crude extracts against *Artemia salina*. The toxicity activity of the brine shrimp test was developed by Micheal, Thompsom, & Abramovitz (1956) and adapted by others (Meyer, Ferrighi, Putnam, Jacobsen, Nichols, & McLaughlin,1982).; Solis, Wright, Anderson, Gupta, & Phillipson, 1993). It is a convenient preliminary toxicity test, since the brine shrimp is highly sensitive to a variety of chemical substances. The assay is considered a very useful tool for preliminary toxicity assessment of plant extract (Solis et al.,1993). Phytochemical screening was also carried out to identify the presence of principal chemical substances of crude hexane, dichloromethane and methanol extracts and the identification of corresponding crude extracts which are responsible for the toxicity activity. It is hoped that this study would contribute to the knowledge and the utilization of *S. polycystum* if the macroalgae species were found to be non-toxic to human.

## 2. MATERIALS AND METHOD

*Sargassum polycystum* was collected from Port Dickson beaches, within 20 - 200 meters offshore. Sampling of *S. polycystum* was carried out at littoral and sub-littoral zones during early months where the low seawater level provide easier condition to collect the samples.

### *2.1 Preparation of Crude Extract*

The samples were cleaned and dried at room temperature for 24 hours. The samples were then extracted with hexane, dichloromethane and methanol by maceration technique at room temperature, filtered and evaporated under reduced pressure to give crude extracts. The dried extracts were stored in glass bottle at $4^0$C for further analysis.

### *2.2 Toxicity Testing*

All the crude extracts were subjected for toxicity test using the brine shrimp lethality (BSL) assay system (Meyer et al., 1982). Stock solutions of the algal extracts were prepared by dissolving 200 mg of each crude extract in 1ml of sterilized distilled water. The mixture was

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vortexed to ensure that the extracts were homogeneous. The working stock solutions were protected from light by covering the bottle with aluminum foil.

### *2.3 Hatching Shrimp*

Brine shrimp eggs, *Artemia salina* L. were hatched in 1L aquarium containing filtered seawater. The aeration system was placed at the bottom of the aquarium to ensure complete hydration of the larvae. After 24 h incubation at room temperature (22°C – 29°C), the larvae were attracted to one side of the vessel with a light source and collected with a pipette. The freshly hatched free-swimming larvae were used for the bioassay.

### *2.4. Brine Shrimp Assay*

The brine shrimp lethality (BSL) assay system was prepared with 3 ml of filtered seawater containing several concentrations of extract. In each tube 10 larvae were transferred and top up with filtered sea water until the final volume reached 5 ml. The setup was allowed to remain under constant illumination for 24 h. After 24 h, the tubes were then examined and the number of dead larvae in each tube was counted and recorded. The results of brine shrimp mortality against the logarithm of concentrations were plotted using the Microsoft Excel computer program and the regression equations were used to calculate $LC_{50}$ value.

### *2.5 Phytochemical Screening*

A portion of the plant sample was subjected to phytochemical screening to test the presence of alkaloid, flavonoid, terpenoid, coumarin, tannin and steroid. The crude extracts were screened for phytochemical constituents for macroalgae secondary metabolites using standard procedures of analysis by Manuel, Balangcod,, Laruan,., Patacsil, & Martin,P.2012).

### *2.6 Data Analysis*

The toxic effect was calculated for each crude extract using the profit analysis method described by Meyer et al. (1982).

$$\text{Percent of Mortality}\,(\%) = \frac{\text{Number of larvae dead}}{\text{Number of larvae}} \times 100\% \qquad (1)$$

The results of brine shrimp mortality against the logarithms of concentration was plotted using Microsoft Excel computer program. The $LC_{50}$ value was obtained by linear regression equations.

$$Y = mX + c \qquad (2)$$

Where:

Y = Log of concentration

X = Figures probit (50% of mortality)

Determine the percentage of mortality,

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$$\text{Mortality}(\%) = \frac{D - C}{10} \times 100\% \quad (3)$$

Where:

D = number of larvae dead under treatment

C = numbers of larvae dead under control

10 = total of larvae used for each treatment

The toxicity level for crude extracts can be determined based on $LC_{50}$ value. According to Meyer et al. (1982), the extract is considered more toxic if $LC_{50}$ value less than 30 $mgml^{-1}$, while toxic if $LC_{50}$ value was 30-1000 $mgml^{-1}$ and not to be toxic if the LC50 value up to1000 mg $ml^{-1}$.

## 3. RESULT AND DISCUSSION

### *3.1 Toxicity Test of S. polycystum*

Toxicity activity of *S. polycystum* crude extracts which exhibited positive results were examined against *Artemia salina.* The results shown in Table 1 indicate the Mean±SD of each treatment concentration. All crude extracts were responsive toward the death of *A.salina* (% ± SD) by which contributed up to 70% of larvae death at the 1000 $mgml^{-1}$ of concentration. The toxicity activity in all extracts of *S.polycystum* stopped at 50 $mgml^{-1}$. Mean value of 50% mortality of *A.salina* for all crude extracts achieved at concentrations ranged from 250 to 500 $mgml^{-1}$ with the value is 46.7±2.5 to 60.0±1.8%. Table 2 showed that all extracts (hexane, dichloromethane and methanol) exhibited positive inhibition. ANOVA analysis showed that 50% of larvae death achieved at low relative concentrations. The $LC_{50}$values for both hexane and dichloromethane extracts were 275.42 $mgml^{-1}$ and methanol extract 295.12 $mgml^{-1}$ which were not significantly different as shown in Figure 1.

In all cases, mortality percentage gradually declines as the extract concentration decreases. It is noted that the extracts have shown a dose-response relationship in which the increase of dose exposure is proportional to the number of dead brine shrimp, as shown in Table 1. This is consistent with Harborne's (1994) finding which states that the increasing of the extract concentration is parallel with the increasing of toxicity level. According to Meyer et al. (1982), an extract is considered toxic if the $LC_{50}$ value of mortality in range 30 -1000 mg $ml^{-1}$, whereas $LC_{50}$ value up to 1000 $mgml^{-1}$ was considered non toxic. In the present study, the $LC_{50}$ values of mortality from all extracts are more than 1000 $mgml^{-1}$, indicating these extracts can be considered non toxic which is consistent with other findings ( Mahsa et al., 2014; Chiao-Wei, Siew-Ling & Ching-Lee, 2011).

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Table 1: Toxicity level of crude extracts from *Sargassum polycystum* against brine shrimp larvae.

| Extraction Solvent | Number of dead larvae (% mortality) | | | | | | |
|---|---|---|---|---|---|---|---|
| Conc. (mg ml $^{-1}$) | 1000 | 750 | 500 | 250 | 100 | 50 | 0 (Control) |
| $Log_{10}$ Conc. | 3 | 2.9 | 2.7 | 2.4 | 2.0 | 1.7 | 0 |
| Hexane | 21<br>70.0±2.4$^{a}$ | 20<br>66.7±2.4$^{a}$ | 18<br>60.0±1.8$^{b}$ | 14<br>46.7±2.4$^{c}$ | 14<br>46.7±2.4$^{c}$ | 10<br>33.3±3.0$^{d}$ | 0<br>0.0±0.0$^{f}$ |
| Dichloro methane | 18<br>60.0±2.9$^{b}$ | 17<br>56.7±2.4$^{b}$ | 14<br>50±2.5$^{c}$ | 14<br>46.7±2.5$^{c}$ | 8<br>26.7±3.0$^{e}$ | 7<br>23.3±3.0$^{e}$ | 0<br>0.0±0.0$^{f}$ |
| Methanol | 21<br>70.0±3.2$^{a}$ | 18<br>60.0±2.4$^{b}$ | 17<br>56.7±2.4$^{b}$ | 14<br>46.7±2.5$^{c}$ | 13<br>43.3±3.5$^{c}$ | 11<br>36.7±3.5$^{d}$ | 0<br>0.0±0.0$^{f}$ |

Different letters indicate significant differences within each treatment according to Duncan's test ($p<0.05$)

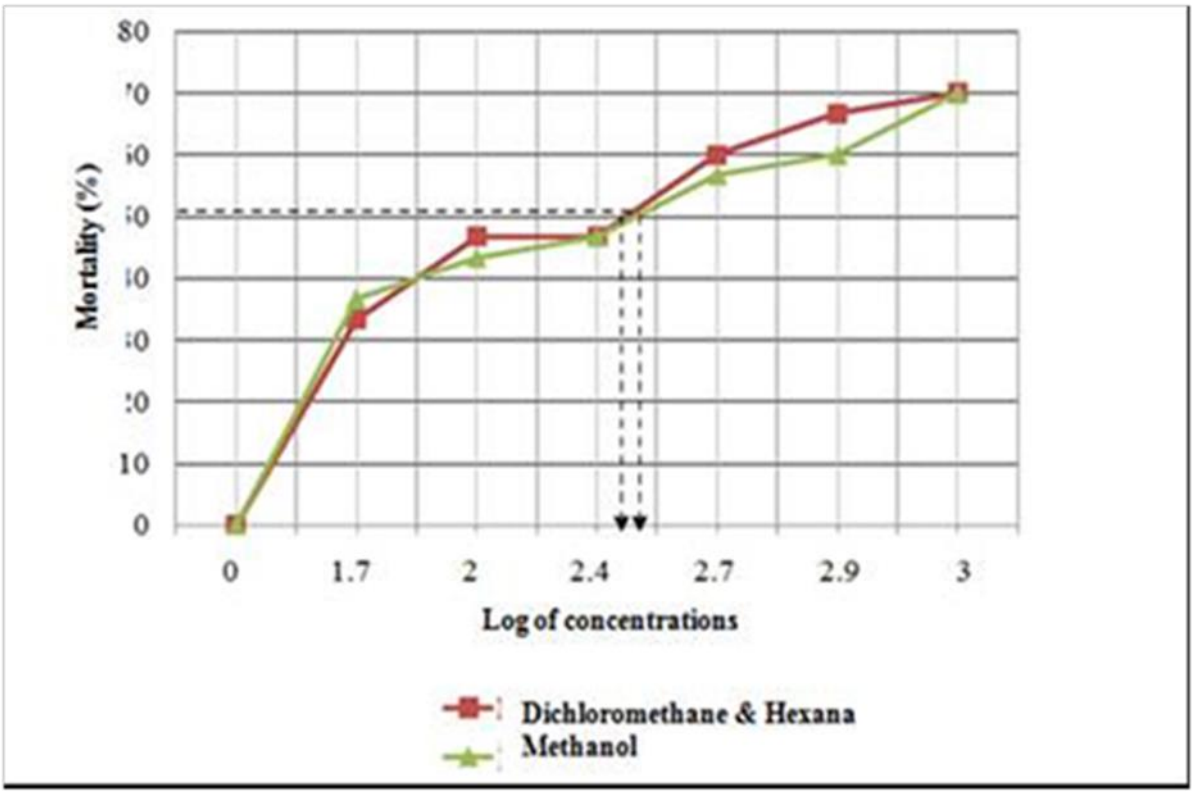


Figure 1: Value Of $LC_{50}$ For *Sargassum polycystum* Crude Extract In Difference Solvents.

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Table 2: Value Of $LC_{50}$ For *SargassumpPolycystum* Crude Extract In Difference Solvents.

| Extraction Solvent | Value of x-axis | Value of $LC_{50}$ ($mgml^{-1}$) |
|---|---|---|
| Hexane | 2.44 | 275.42 |
| Dichloromethane | 2.44 | 275.42 |
| Methanol | 2.47 | 295.12 |

### *3.2 Phytochemical Screening of S. polycystum*

The phytochemical screening of *S. polycystum* showed the occurance of possible bioactive components in different extracts. The qualitative phytochemical analysis exhibited the presence of alkaloid, flavonoid, coumarin, tannin and steroid in *S. polycystum* from all three solvents used to extract the macroalgae. The data were presented in Table 3. Methanol, a polar solvent was found to contain the most chemical constituents in the extraction, while dichloromethane and hexane solvents were able to extract alkaloid and flavonoid with high concentration. In flavonoid test, the higher color intensity was observed in the methanol extract of *S.polycystum* than the dichloromethane and hexane extracts. Dichloromethane is a better solvent for extracting alkaloids from the macroalgae compared with hexane and methanol.

The results in Table 3 showed that alkaloids and flavonoids were the dominant components in all extracts. A wide range of solvent has been proven to be effective in extracting to extract alkaloids and flavonoids from macroalgae. Ethanolic solvent for example, was found to efficiently extract flavonoid from macroalgae of class Phaeophyceae and moderately of class Chlorophyceae and Rhodophyceae (Sarojini, Lakshminarayana, & Seshagiri 2012). Other study has also shown hexane could effectively extract flavonoids from *Kappaphycus alvarezii*, a member of red seaweed, followed by chloroform, ethyl acetate and methanol (Lalopua, Sukoso, & Aulani'am, 2011), which is in contrast to our findings. A study carried out by Siregar, Sabdono, & Pringgenies (2012) demonstrated the efficiency of hexane and methanol to extract alkaloid and flavonoid compounds from genus *Sarggasum*. This is in agreement with our study that showed hexane, dichloromethane and methanol are the most efficient solvents to extract flavonoids and alkaloids from the macroalgae studied.

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Table 3: Phytochemical Screening Of Crude Extracts Of *Sargassum polycystum.*

| Solvent | Bioactive Compound | | | | | |
|---|---|---|---|---|---|---|
| | Alkaloid | Flavonoid | Terpenoid | Coumarin | Tannin | Steroid |
| Hexane | ++ | + | - | + | - | - |
| Dichloromethane | +++ | ++ | - | - | - | - |
| Methanol | + | +++ | - | + | ++ | ++ |

-: Not found; +: Low concentration; ++: Moderate concentration; +++: High concentration

## 4. CONCLUSION

Different toxicity values in Brine Shrimp Lethality (BSL) assay were shown in different *S. polycystum* extractions. $LC_{50}$ values of both hexane and dichloromethane extracts were 275.42 $mgml^{-1}$ and 295.12 $mgml^{-1}$ for methanol extract. All extracts showed no significant inhibition against 50% of the mortality of *A. salina* larvae. The phytochemical screening shows that alkaloids and flavoids were the major chemical constituents found in *S. Polysyctum* extractions. These secondary metabolites are considered non-toxic as they did not show significant toxic response at 1000 mgml-1 concentration. Nevertheless, further investigation for these marine macroalgae extracts is required to identify the active components that could be developed as potential drugs to be used against diseases.

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