

PHYTOL-CONTAINING SEAWEED EXTRACTS AS CONTROL FOR *Ganoderma boninense*

SYAMIMI DIYANA ABDUL AZIZ*; NUR FAZIRAH JAFARAH* and ZETTY NORHANA BALIA YUSOF**

ABSTRACT

Basal stem rot (BSR) is a disease in oil palm caused by a fungal pathogen, *Ganoderma boninense*. Utilisation of seaweeds as a control agent has not been explored. This study investigated the anti-fungal potential of Malaysian seaweed extracts against *G. boninense* and identification of the compounds. Seaweeds of *Sargassum oligocystum*, *Caulerpa racemosa*, *Caulerpa racemosa var. lamouroxii* and *Halimeda macrophysa* were collected and subjected to crude extraction with various solvents. Methanolic extracts of all species displayed the highest yield with an average of 15.25% compared to dry weight. Anti-fungal assays were carried out against *G. boninense* using the poisoned food technique and three highest inhibitions were exhibited by *C. racemosa var. lamouroxii* dichloromethane extract (46.82%), *H. macrophysa* dichloromethane extract (33.49%) and *C. racemosa* methanol extract (28.06%). Dominant compounds detected via gas chromatography-mass chromatography (GC-MS) in extracts with anti fungal potential includes phytol. Anti-fungal assay using standard phytol showed growth inhibition of *G. boninense* of up to 21% inhibition. *Caulerpa racemosa var. lamouroxii*, *H. macrophysa*, *C. racemosa* and *S. oligocystum* dichloromethane extracts contain 474, 117, 106 and 19 mg litre⁻¹ of phytol respectively. These findings suggested that Malaysian seaweeds are a good source of anti-fungal compounds for utilisation in controlling the BSR disease of oil palm in Malaysia.

Keywords: anti-fungal activity, basal stem rot disease, *Ganoderma boninense*, phytol, seaweeds.

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INTRODUCTION

Oil palm is a major source of edible vegetable oil and biodiesel fuel in Malaysia (Hushiarian *et al.*, 2015). However, it is confronted with a devastating disease caused by a fungus, *Ganoderma boninense*. This fungus acts as the main factor that causes basal stem rot (BSR) disease which affects the production of palm oil and finally results in death (Khairudin, 1990; Rao, 1990; Hushiarian *et al.*, 2015). Many

approaches to prevent this disease have been done but the most common method is by applying non-environmental-friendly fungicide which is also costly at the same time (Idris *et al.*, 2002). Therefore, a more environmental-friendly and sustainable remedy to this disease would be worth exploring. Seaweeds or also known as macrolagae are marine plants which have shown to have some fungicidal potential (Demirel *et al.*, 2009). A number of studies have proposed the potential of seaweeds in inhibiting fungal species (Khanzada *et al.*, 2007; Aruna *et al.*, 2010; Manivannan *et al.*, 2011; Stein *et al.*, 2011; Peres *et al.*, 2012; Rajasulochana *et al.*, 2013; Am *et al.*, 2015; Ambika and Sujatha, 2015). For example, a study by Rajasulochana *et al.* (2012) showed *Kappaphycus alvarezii* expressed maximum activity against *Pseudomonas fluorescense*, *Aspergillus*

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fumigatus and *Staphylococcus aureus* but lower inhibition on *Vibrio cholera* and *Proteus mirabilis*. In another study, five brown seaweed extracts namely *Sargassum vulgare*, *Cystoseira barbata*, *Dictyopteris membranacea*, *Dictyota dichotoma* and *Colpomenia sinuosa*, displayed high anti-fungal activities against eight fungal species. Some algal extracts even exhibited anti-fungal activity that is relative to commercial anti-fungal medicine (Am *et al.*, 2015). Seaweeds are abundant in Malaysia and most of their potential and advantages are under-explored. This study aimed to discover the possession of anti-fungal characteristics and identification of the bioactive compounds in the abundantly available Malaysian seaweeds which could be the answer to the quest of an environmental-friendly approach to control the BSR disease in oil palm.

MATERIALS AND METHODS

Collection of Seaweeds

Seaweeds were collected from Teluk Kemang, Port Dickson, Negeri Sembilan, Malaysia (2° 26' N latitude; 101° 51' E longitude). The collected seaweeds were brown seaweed (*S. oligocystum*) and green seaweeds (*C. racemosa* var. *lamouroxii*, *C. racemosa* and *H. macrophysa*). The handling procedures of the collected seaweeds were according to the method by Abirami and Kowsalya (2012) but the samples were freeze-dried using a freeze-dryer for seven days. The seaweeds were then ground into fine powder using a mechanical blender and weighed.

Preparation of Crude Extracts

Ten grams of the seaweed powder was extracted with 250 ml solvent (dichloromethane, methanol and chloroform) via Soxhlet extraction (Abirami, 2012).

Anti-fungal Assay

All of the seaweed extracts were tested using the poisoned food technique (Schmitz, 1930; Bussaman *et al.*, 2012) with the concentration of 0.25, 0.5 and 1.0 mg ml⁻¹ of seaweed extracts. Triadimefon was chosen as positive control due to its ability in inhibiting fungal growth (Jayaratne *et al.*, 2001).

Inhibition percentage of *G. boninense* was measured using the following equation:

$$\% \text{ Inhibition} = \frac{(\text{ADC} - \text{ADT})}{(\text{ADC})} \times 100$$

ADC - average diameter of fungal culture on negative control plate.

ADT - average diameter of fungal culture on plates treated with seaweed extracts.

Identification of Potential Anti-fungal Compounds Using GC-MS

Preparation of methanol extract for solid phase extraction (SPE) was done using the method by Abdullah *et al.* (2014) while the dichloromethane and chloroform extracts were subjected to syringe filtration to remove impurities. Compounds from both extracts were identified using Thermo Scientific TSQ Quantum XLS gas chromatography (GC) (country of origin: USA). The GC capillary column with HP-5MS stationary phase (30 m x 0.25 mm x 0.25 µm) and composed of (5%-phenyl)-methylpolysiloxane was used. The final concentration of crude extracts was prepared to 0.1 g ml⁻¹ by dissolving 0.1 g of the crude extract in 1 ml of solvent used for extraction and mixed well. The ionisation energy used was 70 eV and helium gas (99.999%) was used as carrier gas at constant flow rate of 1.0 ml min⁻¹ and an injection volume of 1 µl (split ratio 10:1). The injection temperature was set at 250°C and ion source temperature at 280°C, the oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C min⁻¹, to 200°C, then 5°C min⁻¹ to 280°C (isothermal for 9 min). Mass spectra were taken at 70 eV (a scan interval of 0.5 s and fragments from 40 to 550 Da). The total GC running time was 36 min. The relative percentage of each component was calculated by comparing its average peak area to the total area. The software used to handle mass spectra and chromatograms was Xcalibur (Upgrade and Bhaskar, 2013).

Anti-fungal Activity of Phytol against *G. boninense*

The anti-fungal activity of phytol against *G. boninense* was determined by poisoned food technique (Schmitz, 1930). The phytol standard (Chemfaces) was prepared using dimethylsulphoxide (DMSO, 1.0% v/v) as the initial solvent carrier followed by dilution with Potato Dextrate Agar (PDA) (at about 50°C) containing final concentration of 100 µg ml⁻¹ antibiotics ampicillin and penicillin to give the desired concentrations of 0.25, 0.5 and 1.0 mg ml⁻¹. The agar was left to solidify, and a 6 mm mycelial disk was cut from the periphery of one-week old cultures, placed in the centre of each PDA plate, and then incubated at 27°C for seven days (Schmitz, 1930; Bussaman *et al.*, 2012).

Quantification of Phytol in Seaweed Extracts

Calibration curve. Calibration curve (peak area versus concentration) was plotted by using the calibration point of phytol standard solution. The compound phytol is quantified by interpolation of the sum of two selected ions peak areas into the

linear plot. In the single-ion monitoring (SIM) mode for quantification, a molecular peak ion for phytol was selected as a quantitative ion (m/z 297). The start time for ion monitoring was programmed from 4.0 to 36.0 min. A pullup delay of 100 ms and an emission current of 70 µA was applied for the ion monitored.

Evaluation of repeatability and reproducibility. The repeatability of the chromatographic analysis was determined in a day by 10 replicates of 1 µl injections of a phytol standard solution at 250 ppm level. The reproducibility for phytol was examined every other day (n = 3) by 1 µl injection of the standard solution at 250 ppm levels.

Data Analysis

Each treatment was replicated three times and the results were expressed as mean ± standard deviation. The median values were subjected to Kruskal-Wallis H tests (SPSS statistical package, version 22) was used to determine the significant differences (p < 0.05) between treatments.

RESULTS AND DISCUSSION

Extraction Yield

Figure 1 shows the extraction yield of the dichloromethane, methanol and chloroform extracts. Three of the different extraction solvents were used per the order of their increasing polarity as different compound gets extracted in different

solvents. Considerable variations in extraction yield were observed among different seaweed species. From 10 g of seaweed powder, methanol extraction relatively produced the highest yield for all seaweed samples. The yield of the extract was obtained by the following equation (Hasmida *et al.*, 2014):

$$\text{Yield (\%)} = \frac{\text{WE}}{\text{WP}} \times 100$$

WE - dry weight of extract.
WP - dry weight of plant powder.

The extraction yield of dichloromethane extracts ranged from 1.19% to 7.85%, methanol extracts ranged from 7.11% to 21.20%, and chloroform extracts ranged from 1.98% to 7.11%. Extraction yield of seaweed extracts was obtained by measuring the weight of crude extracts from Soxhlet extraction. The selection of extraction solvent and method are crucial for extraction of marine compound of interest (Misra *et al.*, 2015). Different solvents have the capacity to solubilise different compounds based on the polarity of the solvent (Hediat and Najat, 2010). In this study, methanol displayed the best extraction capability of most seaweed species compared to the other solvents. This might be due to the polarity of methanol which makes it able to extract not only the polar compounds but also some non-polar compounds that are miscible to methanol such as phenolics, fatty acids, sterols, alkaloids, hydrocarbons and more (Abdel-Aal *et al.*, 2015). Chloroform, on the other hand, is a non-polar solvent which can only solubilise non-polar compounds such as phenolics, diterpene alcohols, fatty acids and other non-polar compounds (Abdel-

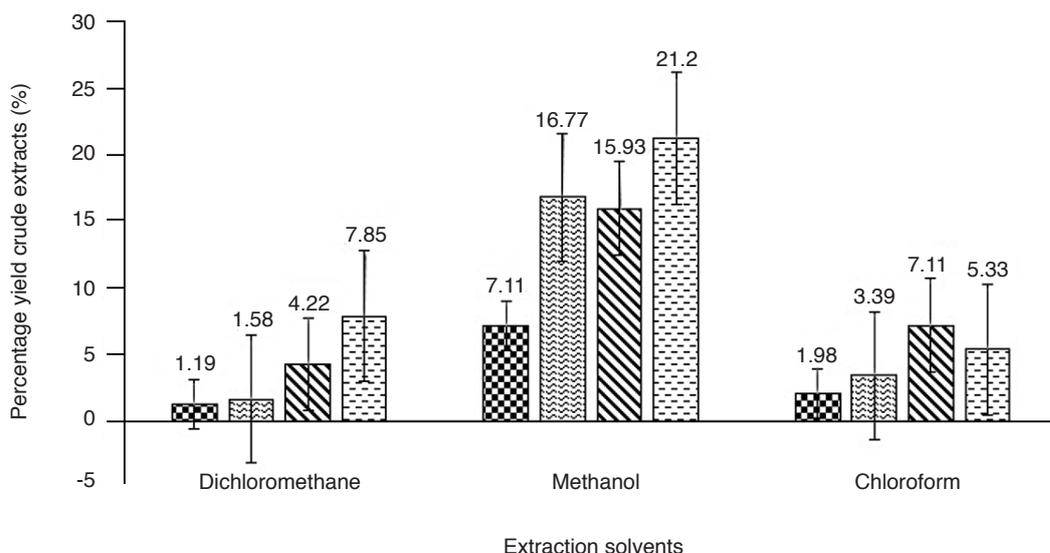


Figure 1. The percentage of extraction yield of the different solvent extracts. Each measure represents average from three replicates per treatment performed in triplicate and the bars represent standard error. Checker board pattern represents *S. oligocystum*; wave pattern represents *C. racemosa*; diagonal stripes pattern represents *C. racemosa var. lamouroxii*; horizontal stripes pattern represents *H. macrophysa*.

Aal *et al.*, 2015). Dichloromethane, however, is a polar solvent which is able to extract only polar compounds.

In vitro Growth Reduction Effect of Seaweed Crude Extracts on *G. boninense*

The anti-fungal activity of seaweed crude extracts from all three solvents against *G. boninense* were studied *in vitro* via poisoned food technique. *Ganoderma boninense* took seven days to completely grow in 9 cm petri plate. This plate acted as control. Based on the observation made on plates containing seaweed crude extracts, some showed significant reduction of *G. boninense* growth at certain concentrations. As can be seen from the graph in Figures 2 to 5, *S. oligocystum* methanol and dichloromethane extracts exhibited optimum anti-fungal activity at the concentration of 0.50 mg ml⁻¹ with 33.02% and 42.02% inhibition but decrease extremely as the concentration increased. For *S. oligocystum* chloroform extract, the anti-fungal activity increased at increasing concentration, and the highest inhibition percentage was 37.83%. Similarly, the anti-fungal activity of *C. racemosa* var. *lamouroxii* methanol and chloroform extracts increased at increasing concentration with inhibition percentage of 30.7% and 33.18% respectively. For *C. racemosa* var. *lamouroxii* dichloromethane extract, the highest activity exhibited at the lowest concentration of 0.25 mg ml⁻¹ with growth reduction of up to 46.82% but reduced with increasing concentration of the extract. *Caulerpa racemosa* methanol extract slightly reduced when the concentration increased. While *C. racemosa* dichloromethane and chloroform extracts demonstrated optimum anti-fungal activity at

concentration of 0.50 mg ml⁻¹ with inhibition 50.08% and 39.33%, respectively. Finally, the anti-fungal activity of *H. macrophysa* for all solvents showed fluctuated inhibition percentage whereby the inhibition percentage was low at concentration of 0.50 mg ml⁻¹ but increased again at concentration of 1.0 mg ml⁻¹.

Marine algae have been reported for various important biological activities such as anti-microbial (Demirel *et al.*, 2009), anti-viral (Newman *et al.*, 2003), anti-inflammation (Ranganayaki *et al.*, 2014), anti-coagulant (Chanda *et al.*, 2010), anti-fouling (Maréchal *et al.*, 2004) and anti-fungal activity (Ambika and Sujatha, 2015; Andreea *et al.*, 2000; Pandian *et al.*, 2011; Saidani *et al.*, 2012). In this study, almost all the algal extracts tested showed anti-fungal activity against *G. boninense* proving that crude extracts of marine algae using various solvents may contain bioactive compounds with potential use. *Caulerpa racemosa* var. *lamouroxii* and *C. racemosa* extracts proved to show good anti-fungal potential while *S. oligocystum* and *H. macrophysa* exhibited low anti-fungal activity against *G. boninense*. A Kruskal-Wallis H test was conducted to explore the effect of solvents and species of extracts towards growth reduction percentage of *G. boninense*. There is statistically significant difference, $r = 21.48$, $p = .00$ in growth reduction percentage between the different solvent with a mean rank growth reduction percentage of 38.63 for solvent dichloromethane, 72.61 for solvent methanol and 52.26 for solvent chloroform. The effect of different seaweed species tested towards growth reduction percentage showed no statistically significant difference, $r = 4.1$, $p = .25$ with a mean rank growth reduction percentage of 58.61 for species *S. oligocystum*, 50.11 for species

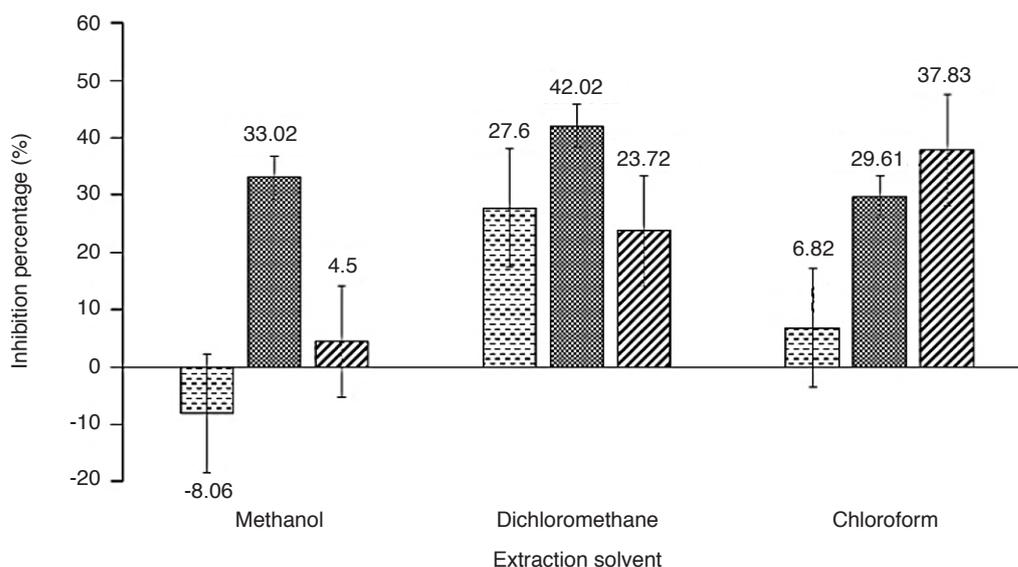


Figure 2. Average *G. boninense* growth reduction of *S. oligocystum* extract concentrations. Each measure represents average from three replicates per treatment performed in triplicate and the bars represent standard error. Horizontal stripes pattern represents 0.25 mg ml⁻¹; small checker board pattern represents 0.50 mg ml⁻¹; diagonal stripes pattern represents 1.0 mg ml⁻¹.

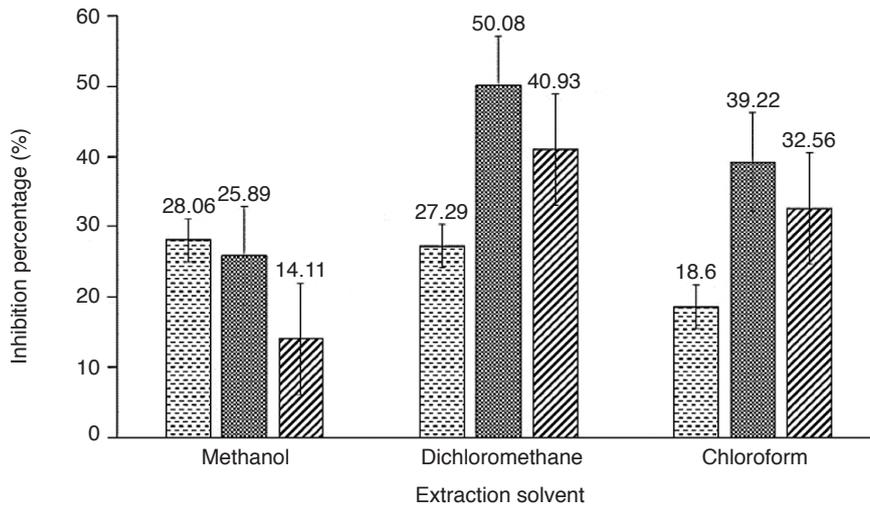


Figure 3. Average *G. boninense* growth reduction of *C. racemosa* extract concentrations. Each measure represents average from three replicates per treatment performed in triplicate and the bars represent standard error. Horizontal stripes pattern represents 0.25 mg ml⁻¹; small checker board pattern represents 0.50 mg ml⁻¹; diagonal stripes pattern represents 1.0 mg ml⁻¹.

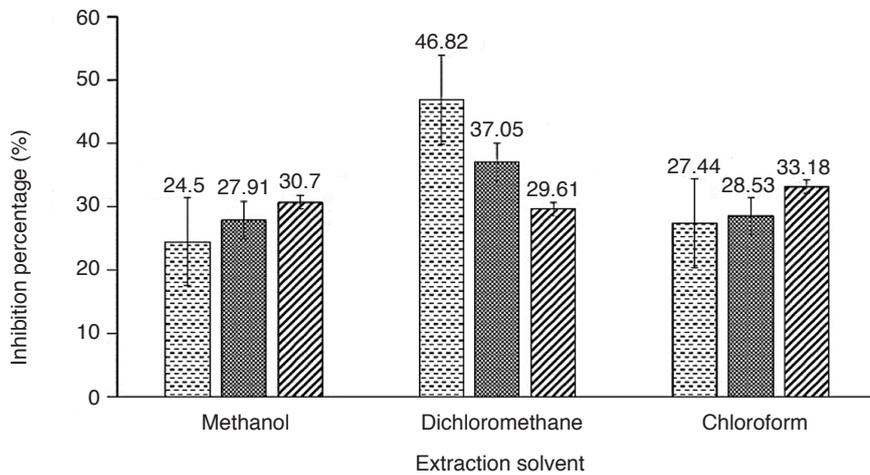


Figure 4. Average *G. boninense* growth reduction of *C. racemosa* var. *lamouroxii* extract concentrations. Each measure represents average from three replicates per treatment performed in triplicate and the bars represent standard error. Horizontal stripes pattern represents 0.25 mg ml⁻¹; small checker board pattern represents 0.50 mg ml⁻¹; diagonal stripes pattern represents 1.0 mg ml⁻¹.

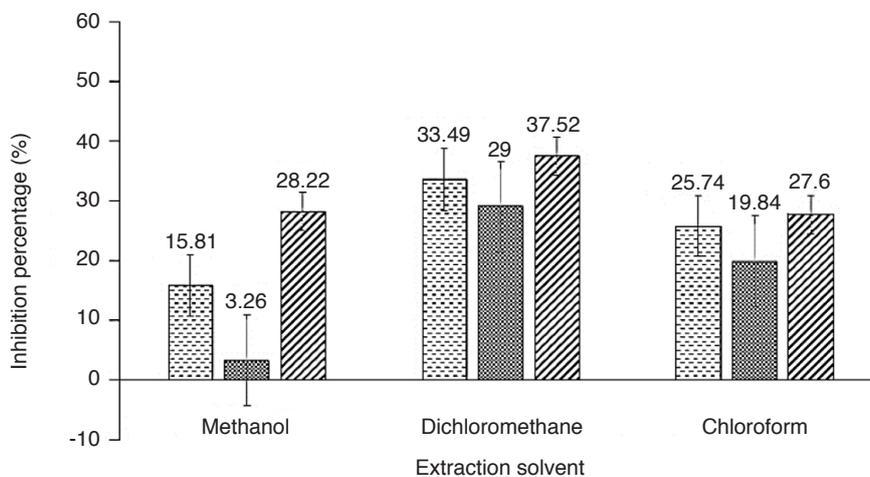


Figure 5. Average *G. boninense* growth reduction of *H. macrophysa* extract concentrations. Each measure represents average from three replicates per treatment performed in triplicate and the bars represent standard error. Horizontal stripes pattern represents 0.25 mg ml⁻¹; small checker board pattern represents 0.50 mg ml⁻¹; diagonal stripes pattern represents 1.0 mg ml⁻¹.

C. racemosa, 47.13 for species *C. racemosa* var. *lamouroxii* and 62.15 for species *H. macrophysa*.

However, there are many factors that should be taken into account namely their habitat, season, and also growth stage (Karthikaidevi *et al.*, 2009).

GC-MS Analysis of Anti-fungal Compounds in Seaweed Extracts

Seaweed extracts with significant anti-fungal activities of above 30% growth reduction at concentration of 0.25 mg ml⁻¹ were further analysed using GC-MS. Compounds from the extracts were identified by comparing the output of the analysis to the National Institute of Standard and Technology (NIST) database. A total of five major compounds were identified in *S. oligocystum* dichloromethane extract as shown in Table 1.

For *C. racemosa* var. *lamouroxii*, there were six major compounds identified in its methanol extract, and five major compounds in its dichloromethane and chloroform extracts as shown in Table 2.

While in *C. racemosa* extract, five major compounds were identified in both methanol and dichloromethane extracts as shown in Table 3.

Finally, there were six major compounds with high probability identified in *H. macrophysa* dichloromethane and chloroform extracts as shown in Table 4.

Based on the results obtained all extracts mainly consist of compound from the classes of phenolic compounds, vitamins, diterpene alcohol, fatty acids and sterols being identified as anti-microbials. Based on this finding, the bioactive compounds identified are expected to cause the inhibitory effect of seaweed extracts towards the growth of *G. boninense* *in vitro*. This includes the presence of phytol (Shobier *et al.*, 2016), 1-(+)-ascorbic acid 2,6-dihexadecanoate (Karthikeyan *et al.*, 2014), linolenic acid, linoleic acid (Raynor *et al.*, 2004) and ethyl iso-allocholate (Tay and Chong, 2016) which are correlated with strong biological activity. Phytol was one of the compounds identified in most potential extracts and was tested alone with two concentrations, 0.25 and 0.50 mg ml⁻¹

TABLE 1. CHEMICAL CONSTITUENTS OF *S. oligocystum* EXTRACTS

| Extracts | RT (min) | Component name | Molecular formula | MW (m/z) | Quality |
|------------------|----------|---|--|----------|---------|
| Dichloro-methane | 11.17 | 2-Decen-1-ol | C ₁₀ H ₂₀ O | 156 | 12.60 |
| | 12.70 | Hexadecanal, 2-methyl- | C ₁₇ H ₃₄ O | 254 | 13.09 |
| | 14.22 | n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 68.55 |
| | 15.23 | Z,Z,Z-1,4,6,9-Nonadecatetraene | C ₁₉ H ₃₂ | 260 | 17.03 |
| | 33.64 | Cholesta-8,24-dien-3-ol,4-methyl-, (3 α ,4 α)- | C ₂₈ H ₄₆ O | 398 | 15.08 |

Note: MW - molecular weight. RT - retention time.

TABLE 2. CHEMICAL CONSTITUENTS OF *C. racemosa* var. *lamouroxii* EXTRACTS

| Extracts | RT (min) | Component name | Molecular formula | MW (m/z) | Quality |
|------------------|----------|---|---|----------|---------|
| Methanol | 14.25 | 1-(+)-Ascorbic acid 2,6-dihexadecanoate | C ₃₈ H ₆₈ O ₈ | 652 | 29.76 |
| | 16.29 | Phytol | C ₂₀ H ₄₀ O | 296 | 22.82 |
| | 19.64 | Ethyl iso-allocholate | C ₂₆ H ₄₄ O ₅ | 436 | 40.43 |
| | 20.10 | Ergosta-5,22-dien-3-ol, acetate, (3 α ,22E)- | C ₃₀ H ₄₈ O ₂ | 440 | 28.81 |
| | 21.01 | 9-Bromo-10-[2-pyridylamino]-deoxoartemisinin | C ₂₀ H ₂₇ BrN ₂ O ₄ | 438 | 26.18 |
| | 22.03 | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester | C ₃₅ H ₆₈ O ₅ | 568 | 43.02 |
| Dichloro-methane | 11.71 | Tetradecanoic acid | C ₁₄ H ₂₈ O ₂ | 228 | 49.29 |
| | 14.29 | 1-(+)-Ascorbic acid 2,6-dihexadecanoate | C ₃₈ H ₆₈ O ₈ | 652 | 61.59 |
| | 16.30 | Phytol | C ₂₀ H ₄₀ O | 296 | 60.46 |
| | 17.78 | Phytol, acetate | C ₂₂ H ₄₂ O ₂ | 338 | 24.30 |
| | 19.50 | 10,12,14-Nonacosatriynoic acid | C ₂₉ H ₄₆ O ₂ | 426 | 34.44 |
| Chloroform | 16.29 | Phytol | C ₂₀ H ₄₀ O | 296 | 52.62 |
| | 17.25 | Hexadecanamide | C ₁₆ H ₃₃ NO | 255 | 64.74 |
| | 19.91 | Androst-4-en-11-ol-3,17-dione, 9-thiocyanato- | C ₂₀ H ₂₅ NO ₃ S | 359 | 33.04 |
| | 20.36 | 18,3-Epoxy pregn-9(11)-en-20-one, 3 α -methoxy-(5 beta)- | C ₂₂ H ₃₂ O ₃ | 344 | 28.12 |
| | 20.41 | 4a,10a-Methanophenanthren-9 α -ol, 11-syn-bromo-1,2,3,4,4a,9,10,10a-octahydro- | C ₁₅ H ₁₇ BrO | 292 | 43.72 |

Note: MW - molecular weight. RT - retention time.

TABLE 3. CHEMICAL CONSTITUENTS OF *C. racemosa* EXTRACTS

| Extracts | RT (min) | Component name | Molecular formula | MW (m/z) | Quality |
|------------------|----------|---|--|----------|---------|
| Methanol | 7.59 | 1,1'-Biphenyl, 4-methyl- | C ₁₃ H ₁₂ | 168 | 17.19 |
| | 14.21 | l-(+)-Ascorbic acid 2,6-dihexadecanoate | C ₃₈ H ₆₈ O ₈ | 652 | 55.48 |
| | 16.31 | Phytol | C ₂₀ H ₄₀ O | 296 | 23.24 |
| | 16.74 | Linolenic acid | C ₁₈ H ₃₀ O ₂ | 278 | 34.20 |
| | 21.03 | 9,12,15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl)oxy] propyl ester, (Z,Z,Z)- | C ₂₇ H ₅₂ O ₄ Si ₂ | 496 | 36.02 |
| Dichloro-methane | 9.93 | Trans-2-undecenoic acid | C ₁₁ H ₂₀ O ₂ | 184 | 13.29 |
| | 14.39 | n-Hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 54.67 |
| | 16.84 | 10-Undecyn-1-ol | C ₁₁ H ₂₀ O | 168 | 14.57 |
| | 25.83 | 8-Methyl-6-nonenamide | C ₁₀ H ₁₉ NO | 169 | 24.10 |
| | 31.58 | á-Tocopherol, O-methyl- | C ₂₉ H ₅₀ O ₂ | 430 | 21.81 |

Note: MW - molecular weight. RT - retention time.

TABLE 4. CHEMICAL CONSTITUENTS OF *H. macrophysa* EXTRACTS

| Extracts | RT (min) | Component name | Molecular formula | MW (m/z) | Quality |
|------------------|----------|---|--|----------|---------|
| Dichloro-methane | 11.73 | Tetradecanoic acid | C ₁₄ H ₂₈ O ₂ | 228 | 55.16 |
| | 16.32 | Phytol | C ₂₀ H ₄₀ O | 296 | 68.57 |
| | 17.01 | Octadecanoic acid | C ₁₈ H ₃₆ O ₂ | 284 | 38.36 |
| | 20.47 | 2-[4-methyl-6-(2,6,-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde | C ₂₃ H ₃₂ O | 324 | 39.61 |
| | 26.49 | Ethyl iso-allocholate | C ₂₆ H ₄₄ O ₅ | 436 | 31.23 |
| Chloroform | 31.49 | 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol | C ₂₇ H ₄₆ O | 386 | 41.12 |
| | 31.72 | Vitamin E | C ₂₉ H ₅₀ O ₂ | 430 | 30.03 |
| | 13.49 | ç-Sitosterol | C ₂₉ H ₅₀ O | 414 | 78.74 |
| | 14.27 | l-(+)-Ascorbic acid 2,6-dihexadecanoate | C ₃₈ H ₆₈ O ₈ | 652 | 63.43 |
| | 16.30 | Phytol | C ₂₀ H ₄₀ O | 296 | 59.22 |
| Chloroform | 26.32 | Ethyl iso-allocholate | C ₂₆ H ₄₄ O ₅ | 436 | 33.94 |
| | 31.45 | Cholesterol | C ₂₇ H ₄₆ O | 386 | 44.83 |
| | 34.72 | Pentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)]octacos-1(25),3,5,7(28),9,11,13(27),15,17,19 (26),21,23-dodecaene-25,26,27,28-tetrol, 5,11,17,23-tetrakis (1,1-dimethylethyl) | C ₄₄ H ₅₆ O ₄ | 648 | 45.60 |

Note: MW - molecular weight. RT - retention time.

against *G. boninense* by using chemical standard via poison food technique showed positive results as shown in Figure 6.

The application of chemical standard phytol against *G. boninense* has shown significant anti-fungal activity from the two concentrations tested.

Phytol Quantification of Seaweed-dichloro-methane Extracts via GC-MS

Quantification of phytol content in the four seaweed-dichloromethane extracts that consistently exhibited high anti-fungal activities were carried out via GC-MS. Figure 7 shows that phytol was found to be abundant in *C. racemosa* var. *lamouroxii* extract followed by *H. macrophysa*, *C. racemosa* and *S. oligocystum*.

Phytol is known as product of chlorophyll metabolism in plants (Knaff, 1991) and was proven

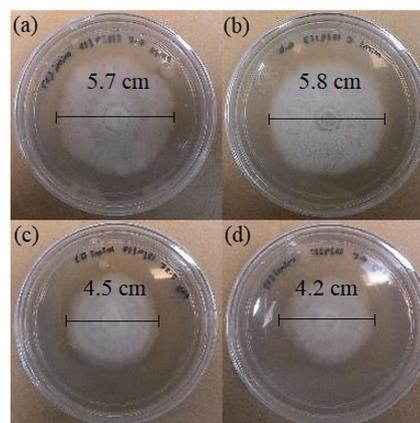


Figure 6. Anti-fungal assay of chemical standard phytol against *G. boninense* after seven days. (a) Negative control consists of agar associated with 1% dimethyl-sulphoxide (DMSO). (b) Absolute control consist of agar without any solvent. (c) Agar associated with 0.25 mg ml⁻¹ concentration of phytol. (d) Agar associated with 0.50 mg ml⁻¹ concentration of phytol.

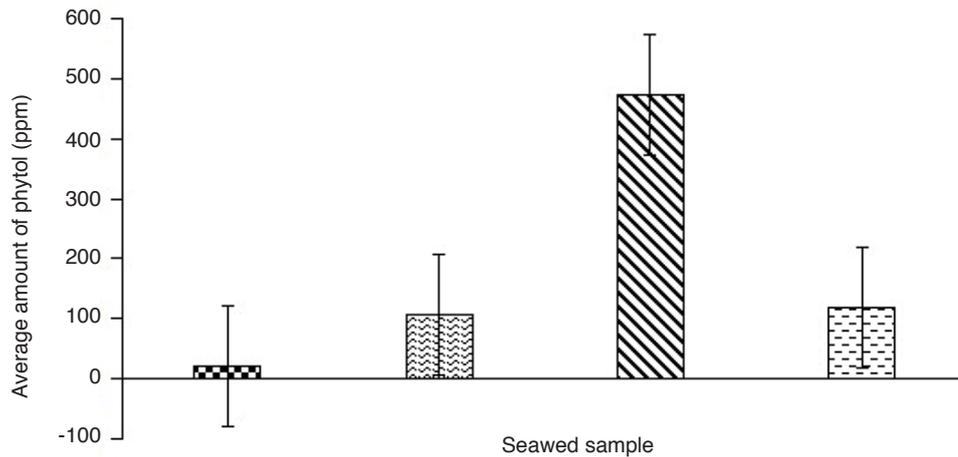


Figure 7. Calculated amount of phytol in seaweed-dichloromethane extracts. Each measure represents average from three replicates and the bars represent standard error. Checker board pattern represents *S. oligocystum*; wave pattern represents *C. racemosa*; diagonal stripes pattern represents *C. racemosa var. lamouroxii*; horizontal stripes pattern represents *H. macrophysa*.

as an anti-microbial compound (Plaza *et al.*, 2010; Ghaneian *et al.*, 2015). Phytol possesses antibacterial activities against *Staphylococcus aureus* by damaging the cell membrane and causing leakages of potassium ions in the bacterial cells (Inoue *et al.*, 2005). Phytol was found in high amount in *C. racemosa var. lamouroxii* extract which explained why the extract portrayed the highest inhibition percentage followed by *H. macrophysa* extract which also corresponded to its anti-fungal activity. Phytol was more concentrated in *C. racemosa* extract than in *S. oligocystum* extract even though *S. oligocystum* extract exhibited higher inhibition percentage. It is suggested that there might be another anti-fungal compound in *S. oligocystum* extract that caused it to be more anti-fungal than *C. racemosa* extract. Further detailed quantification and compound identification of all the extracts that portrayed positive anti-fungal activity will definitely pave the way for the discovery of seaweeds as a source of useful anti-fungal compounds for various purposes.

CONCLUSION

In this study, it was found that the solvent methanol produced the highest crude seaweed extract yield. *Caulerpa racemosa var. lamouroxii* extract showed the best activity against *G. boninense* compared to other species tested. Seaweed-dichloromethane extracts consistently showed significant amount of inhibition against *G. boninense* at the lowest concentration tested which was 0.25 mg ml⁻¹ suggesting that more anti-fungal compounds were extracted through this solvent. GC-MS analysis showed that phytol is one of the dominant compounds identified in the seaweed-dichloromethane extracts and was proven to contribute to the reduction of growth of *G. boninense* based on the assay using a standard. These

findings will pave the way for further exploration of local seaweeds for bioactive compounds which could be useful for various purposes.

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