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Gas chromatography-mass spectrometry analysis of phytocomponents of *sargassum polycystum*

Nurul Najwa Zamimi[1], Noorhazayti Ab. Halim[*1], Deny Susanti Darnis[2], Widya Lestari[3], Md Muziman Syah Md Mustafa[4]

[1]Department of Paediatric Dentistry & Dental Public Health, Kulliyyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia
[2]Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, Kuantan, Pahang, Malaysia
[3]Department of Oral Biology, Kulliyyah of Dentistry, International Islamic University Malaysia, Kuantan, Pahang, Malaysia
[4]Department of Optometry and Visual Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

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ABSTRACT

Sargassum polycystum is a brown seaweed, which has been reported to contain various phytoconstituents especially antimicrobial properties. However, the study on phytochemical components and biological activities of *S. polycystum* are yet fully understood. Therefore, the objectives of this study are to evaluate the best extraction solvents for *S. polycystum* and to determine the percentage of phytocomponents obtained in the *n*-hexane, dichloromethane (DCM) and methanol extracts of *S. polycystum* via Gas Chromatography-Mass Spectrometry (GC-MS) analysis. *S. polycystum* was collected from the coastal area of Sabah, Malaysia. After collection, it was rinsed, dried and extracted with n-hexane, DCM and methanol by soxhlet extraction. The fatty acid compounds (FAME) analysis was done using The Perkin Elmer Turbo Mass Spectrometer. This study showed that methanol is the most efficient solvent as it produced the highest extraction yield with 3.83%, followed by *n*-hexane with 1.69% and lastly DCM with 0.59% in *S. polycystum*. On top of that, this study has found 19 phytocomponents in all extraction methods of *S. polycystum*, which have been proven to possess antibacterial constituents such as palmitic acid, myristic acid, oleic acid, pentadecanoic acid and behenic acid as assessed by GC-MS analysis.

*Corresponding Author

Name: Noorhazayti Ab. Halim
Phone: +609-5705523
Email: zetty@iium.edu.my

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INTRODUCTION

Sargassum polycystum is a brown seaweed originating from the Phaeophyta group (Zailanie *et al.*, 2003). This Sargassum species spreads along the coastal areas from the beach to the coral reefs (Saraswathi *et al.*, 2003). Coral reef is an great area for the seaweed to develop as the thallus could stick firmly from its substratum so that its substrate does not released from the thallus quickly (Kadi, 2005). They are perennial growing throughout the year but strongly depend on the weather as well as the season (Saraswathi *et al.*, 2003). This seaweed

reproduces through the production of spores or a portion of the thallus (Rao *et al.*, 2014).

Phytochemicals are biologically active compounds which are naturally produced by plants and able to connect with one or more living tissue elements that have a broad variety of biological effects (Guaadaoui *et al.*, 2014). Previous studies have revealed that the brown seaweed contains vitamins, free amino acids like omega-3-fatty acid, phenolic compound, essential minerals, mannitol, glucitols, phlorotannins and sulphated polysaccharides (Srivastava and Kulshreshtha, 1989; Wong *et al.*, 2000; Seal and Mathers, 2001; Blunt *et al.*, 2003; Hall *et al.*, 2006). In brown algae, these compounds have a broad range of biological characteristics, such as antioxidant and anticoagulation (Barrow and Shahidi, 2007). One of the brown seaweed compound that has been extensively studied is fucoidan (fucose-containing sulfated polysaccharide) which exhibit many biological and pharmacological properties like anticoagulant/antithrombotic, antitumor, antivirus, and anti-inflammatory effects (Li *et al.*, 2008).

Other than that, the fatty acid compound in brown seaweed have also started to gain researchers attention as it proved to exhibit antibacterial properties (Zheng *et al.*, 2005). The key components of antibacterial properties that inhibit the growth of unnecessary microorganisms are fatty acids (Freese *et al.*, 1973; Zheng *et al.*, 2005). The synthesis of fatty acids in microbes is crucial for the development of a number of lipid-containing elements such as the cell membrane. A previous study had proposed that unsaturated fatty acid like linoleic acid exhibited its antibacterial properties by selectively inhibiting bacterial enoyl-acyl carrier protein reductase (Fab I), an crucial component of the synthesis of bacterial fatty acid (Zheng *et al.*, 2005). This was supported by further studies which showed that fatty acid has important phytopharmaceutical potentials which contributed to its antibacterial properties (Desbois and Smith, 2010). However, the study on phytochemical components and the biological activity of *S. polycystum* has not been extensively investigated. Therefore, this study aimed to evaluate the best extraction solvents for *S. polycystum* and to determine the percentage of phytocomponents in the *n*-hexane, dichloromethane (DCM) and methanol extracts of *S. polycystum* via GC-MS analysis.

MATERIALS AND METHODS

Sample collection and preparation of extract

Samples of *S. polycystum* were collected from the coastal area of Sabah, Malaysia in June, 2017. They were cleaned and freeze-dried for one week. The dried seaweeds were ground into a fine powder and sieved to get a uniform powder size. 100 g of seaweeds powder were successively collected by soxhlet apparatus using various organic solvents; *n*-hexane (68°C), DCM (39.6°C), and methanol (64.7°C) with analytical reagent (AR) quality, based on technique used by Sadasivam and Manickam (1996). To ensure the completion of the extraction process, a comprehensive extraction of each solvent was carried out for six hours. The extraction product of different organic solvents was then evaporated using a rotary evaporator to produce the crude extracts. The resultant crude extracts were then weighed and the percentage of yield was determined as follows,

$$Percentage\ yield\ (\%) = \frac{Extract\ weight\ (grams)}{Sample\ weight\ (grams)} \times 100$$

Results have been shown as a proportion of seaweed dry weight (DW). Before being used in GC-MS analysis, the extracts were held under vacuum desiccators.

Fatty Acid Methyl Ester (FAMEs) preparation

The presence of fatty acid in seaweeds was analysed through GC-MS after crude extracts were converted into methyl esters. BF_3/MeOH (14% boron trifluoride methanol hydroxide (MeOH)) was used to derivate fatty acid to fatty acid methyl esters (FAME). The preparation of FAME was adopted from. In this test, 7 ml of methanolic boron trifluoride solution was pipetted through the top condenser to mix it together with the extracts. The condenser was attached to the flask and for two minutes the mixture was boiled. Then, through the top of the condenser, 5 ml of heptane was added to the boiling mixture and boiling proceeded for another minute. After that, the mixture was cooled at room temperature and the condenser was removed. An adequate saturated sodium chloride solution was then added to the flask to bring the level of liquid into the neck of the flask, then swirled gently for several times. The top layer (heptane solution) was transferred into a test tube and anhydrous sodium sulphate (Na_2O_4S) was added to remove any traces of water. This solution which contained approximately 100 mg/ml of methyl esters was injected directly into the column for gas-chromatography analysis.

Identification of Bioactive Compound through GC-MS Analysis

GC-MS was used to separate and identify the chemical substituent which was mostly volatile and non-polar compounds. FAME analysis of *S. polycystum* extracts were performed using GC-MS Clarus^{TM} SQ 8

Table 1: Extraction yield of extracts

Seaweeds	Solvent	Weight of dry seaweed(g)	Percentage of yield (% w/w)
S. polycystum	*n*-hexane	100	1.69
	DCM	100	0.59
	methanol	100	3.83

Table 2: GC-MS analysis of *n*-hexane extract of S. *polycystum*

Peak No.	Retention Time (min)	Peak Area (%)	Molecular Formula	Compound	Biological Activities
1	28.12	5.23	$C_{12}H_{24}O_2$	Lauric acid	Antibacterial (Zheng *et al.*, 2005)
2	33.79	3.60	$C_{15}H_{30}O_2$	Myristic acid	Antibacterial (Zheng *et al.*, 2005)
3	38.37	1.82	$C_{17}H_{32}O_2$	Palmitoleic acid	Antimicrobial (Huang *et al.*, 2010)
4	38.96	18.02	$C_{16}H_{32}O_2$	Palmitic acid	Antibacterial (Zheng *et al.*, 2005)
5	43.00	8.44	$C_{19}H_{36}O_2$	Oleic acid	Antibacterial and antifungal (Agoramoorthy *et al.*, 2007)
6	43.63	2.23	$C_{17}H_{34}O_2$	Heptadecanoic acid	Antibacterial (Zheng *et al.*, 2005)
7	47.30	1.75	$C_{20}H_{40}O$	Phytol	Cholesterol-raising fatty acid in human (Cater and Margo, 2001)

Perkin Elmer system (USA). The GC-MS instrument applied the following procedures: Column Elite-5MS fused silica capillary column (30 mm length x 0.25 mm inner diameter x 0.25 μm film thickness, composed of 100% dimethyl polysiloxane), operating in 70 ev electron impact mode (ionizing energy); Helium (99.99%) was used as carrier gas at a constant flow rate of 1 ml/min and 2 μl injection volume (Split ratio of 10:1); The injector temperature was 250 ° C; ion source temperature was 280 ° C. The temperature of the oven was set from 70 ° C (six minutes isothermal) to 280 °C (6 °C / min). At 70 ev, mass spectrum was taken; a scanning interval of 0.5 seconds and fragments from 45 to 450 Da. The complete running time of the GC was 60 minutes. The composition of fatty acid was demonstrated as a percentage of the compound peak areas produced by the Turbo Mass GC- MS software.

RESULTS AND DISCUSSION

Table 1 showed that the DCM extract of *S. polycystum* gave the lowest percentage of extract (0.59%) while methanol extract produced the highest extraction yield (3.83%) while *n*-hexane produced 1.69% yield. Our results suggested that methanol is the best solvent to produce the highest yield of *S. polycystum* extract. Due to their effectiveness, convenient and broad applicability, solvent extraction techniques are often used to prepare extracts from plant products. The output of chemical extraction is determined by extraction time and temperature, pH level, the type of solvents with different

Table 3: GC-MS analysis of DCM extract of S. *polycystum*

Peak No.	Retention Time (min)	Peak Area (%)	Molecular Formula	Compound	Biological Activities
1	14.50	3.26	$C_{15}H_{30}O_2$	Myristic acid	Antibacterial (Zheng *et al.*, 2005)
2	16.07	1.75	$C_{15}H_{30}O_2$	Phytol	Antimicrobial, and antiradical activities (Pejin *et al.*, 2014)
3	16.30	4.85	$C_{17}H_{32}O_2$	Palmitoleic acid	Antimicrobial (Huang *et al.*, 2010)
4	16.51	27.77	$C_{16}H_{32}O_2$	Palmitic acid	Antibacterial (Zheng *et al.*, 2005)
5	18.14	10.51	$C_{18}H_{36}O_2$	Oleic acid	Antibacterial activity (Mcgaw *et al.*, 2002)
6	18.39	2.23	$C_{17}H_{34}O_2$	Octade-canoic acid	Antibacterial (Zheng *et al.*, 2005)
7	21.66	1.40	$C_{20}H_{40}O$	Docosanoic acid	Cholesterol-raising FA in human (Cater and Margo, 2001)

Table 4: GC-MS analysis of methanol extract of S. *polycystum*

Peak No.	Retention Time (min)	Peak Area (%)	Molecular Formula	Compound	Biological Activities
1	14.46	6.54	$C_{15}H_{30}O_2$	Myristic acid	Antibacterial (Zheng *et al.*, 2005)
2	16.27	1.30	$C_{17}H_{32}O_2$	Palmitoleic acid	Antimicrobial (Huang *et al.*, 2010)
3	16.49	52.62	$C_{16}H_{32}O_2$	Palmitic acid	Antibacterial (Zheng *et al.*, 2005)
4	18.12	14.22	$C_{18}H_{36}O_2$	Vaccenic acid	Unknown
5	18.36	4.46	$CH_3(CH_2)_{16}COOH$	Stearic Acid	Antibacterial (Zheng *et al.*, 2005)

polarities, sample-to-solvent ratio, chemical composition, sample physical features and the presence of noise particles (Dai and Mumper, 2010; Do *et al.*, 2014). Via the solvent extraction procedures, *n*-hexane was used to attract non-polar compounds, DCM to extract semi-polar compounds and methanol to extract polar compounds. Previous studies have shown that, out of all solvents, methanol is more effective in extracting low molecular weight polyphenols which mainly consists of polar compounds (Dai and Mumper, 2010). Thus, it can be inferred that seaweeds was mainly composed of lower molecular weight polyphenols since all extracts showed the highest percentage of yield when extracted with methanol as compared to *n*-hexane and DCM.

The GC-MS analysis of *n*-hexane *S. polycystum* crude extract demonstrated the presence of a fatty acid mixture. A total of 19 peaks with different retention times were observed as shown in Tables 2 and 3 and Table 4. The molecular formula and molecular weight for the identified compounds were fetched from the National Institute of Standard and Technology (NIST) library in the GC-MS systems. Phytochemicals in the *n*-hexane, DCM, and methanol extracts of *S. polycystum* elucidated by GC-MS analysis were classified into different groups as fatty acids and terpenes.

Analysis of *n*-hexane extract by GC-MS has shown the existence of palmitic acid (18.02%) as a primary compound, followed by oleic acid (8.44%), lauric acid (5.23%), myristic acid (3.60%), hep-

tadecanoic acid (2.23%), palmitoleic acid (1.82%) and phytol (1.75%) (Table 2). In S. polycystum, lauric acid, palmitic acid, myristic acid and oleic acid were stated to possess antimicrobial activities, which describe the potential of S. polycystum extract as anti-cariogenic agent (Mcgaw *et al.*, 2002). On the other hand, DCM extract exhibited palmitic acid as a major compound (27.77%), followed by oleic acid (10.51%), palmitoleic acid (4.85%), stearic acid (4.59%), myristic acid (3.26%), docosanoic acid (1.40%), and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.73%) (Table 3). For methanol extract, palmitic acid (52.62%) was mostly found in this extract, followed by 11-octadecanoic acid (14.22%), myristic acid (6.54%), stearic acid (4.46%), and palmitoleic acid (1.30%) as shown in Table 4.

This study revealed that the extraction of *S. polycystum* with different solvents produced a large percentage of palmitic acid. The results also showed that all extracts contained palmitic acid, myristic acid and stearic acid which previously have been proven to have antibacterial properties (Zheng *et al.*, 2005). The findings achieved are also consistent with the Sabah coastal research on brown algae fatty acid content, in which palmitic acid was also found to be its major component (Bakar *et al.*, 2017).

In this study, 19 phytocomponents were found in all three types of extraction which are contrary to the findings by Dhamotharan (2002), which only found 9 fatty acids. The variation of fatty acid content in S. polycystum could be caused by variables including place, temperature, weather, growth conditions, collection time, region of the thallus, pollution and epiphytic organisms (Taskin *et al.*, 2007).

All *S. polycystum* extracts in this study possess fatty acids which potentially could be developed into antibacterial agent. This study justified the claimed usage of seaweeds in traditional medicine to treat cariogenic bacteria. Nevertheless, further studies are required in order to fully explore the potential of crude extracts as the antibacterial agents. As a recommendation, future studies on isolation, fractionation and structure elucidation of antibacterial active constituents from the seaweed are required in order to fully grasp the knowledge behind the antibacterial activity of seaweeds. This would provide better results as individual compounds with specific antibacterial activities could work as targeted therapies. Hence, the results obtained from this study could provide useful knowledge and information on seaweed for further pharmaceutical development.

CONCLUSION

Our study concluded that methanol is the best extraction solvent for *S. polycystum* as it produced the highest extraction yield compared to *n*-hexane and DCM solvent. All extracts of *S. polycystum* contained palmitic acid, myristic acid and stearic acid which has been reported to exhibit antibacterial properties.

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