様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２５ 日現在

機関番号：１０１０１
研究種目：若手研究
研究期間：2022～2023
課題番号：２２Ｋ１４８５０

研究課題名（和文）Unrevealing the anti-obesity effects of wakame associated to sphingomyelin synthase inhibition

研究課題名（英文）Unrevealing the anti-obesity effects of wakame associated to sphingomyelin synthase inhibition

研究代表者

ＤＩＶＹＡＶＡＮＩ（Gowda, Divyavani）

北海道大学・保健科学研究院・助教

研究者番号：４０８７５９５２

交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：本研究では、HeLa 細胞を用いたスフィンゴミレイン合成酵素(SMS)阻害スクリーニング法を、標的液体クロマトグラフィー/ 質量分析法を用いて確立することに成功した。次に、ワカメエキスのSMS阻害についてスクリーニングを行った。その結果、サブフラクション7を含むワカメヘキサンエキスにSMS阻害活性が認められた。さらに、ヘキサン画分7に含まれる活性化合物をカラムクロマトグラフィーで精製し、NMRおよびHRMSの特性解析により脂肪酸WH73と同定した。 精製された化合物WH73は、SMS1とSMS2の両方に対してそれぞれ良好な阻害作用を示した。

研究成果の学術的意義や社会的意義
Obesity is a leading risk factor for premature death worldwide. Dietary seaweed have a positive role in controlling the obesity, but the exact mechanisms are unknown. In this study, we have revealed these connections by identifying the active component of wakame that can act on lipid metabolism.

研究成果の概要（英文）：In this research study, we have successfully established a sphingomylein synthase(SMS) inhibition screening assay using HeLa cells by a targeted liquid-chromatography/mass spectrometry. The method establishment results were published in a peer-reviewed international journal. Then, the wakame extract was screened for SMS inhibition. The results showed the wakame hexane extract with subfraction 7 was found to be active for SMS inhibition. Further, the active compound in hexane fraction 7 was purified by column chromatography and identified as a fatty acid WH73 by NMR and HRMS characterizations. Purified compound WH73 showed good inhibition for both SMS1 and SMS2 respectively. We are now performing the in vitro results of WH73 using fatty liver cell model. Overall, the possible compound from wakame that should have a possible role in obesity control associated with lipid metabolism was reveled.

研究分野：lipid chemistry

キーワード：Sphingomyelin synthase　wakame　LC-MS　fatty acid　fatty liver　obesity　NMR　chromatography

様　式　Ｃ－１９、Ｆ－１９－１（共通）

１．研究開始当初の背景

Obesity is a metabolic disorder characterized by increased body weight and excessive fat accumulation, which causes high health risks. Rates of overweight and obesity continue to grow in adults and children. From 1990 to 2022, the percentage of children and adolescents aged 5–19 years living with obesity increased four-fold from 2% to 8% globally, while the percentage of adults 18 years of age and older living with obesity more than doubled from 7% to 16%. Worldwide, at least 2.8 million people die each year because of being overweight or obese, and an estimated 35.8 million are suffering from obesity-associated disorders. In, Japan about 33 % of adults are estimated to be overweight or obese. This percentage is significantly low compared to other countries such as Mexico (75%) and the United States (71%). The possible reason for this low is supposed to be the traditional dietary habits of the Japanese population. In particular, seaweed consumption is reported to have potential therapeutic benefits in the management of body weight and obesity. It is known that an imbalance of sphingolipid metabolism is associated with fatty liver disease and obesity. An enzyme that converts ceramide to sphingomyelin (SM) named sphingomyelin synthase (SMS1 or SMS2) is known to be the main target and its inhibition has been reported to have promising therapeutics to control obesity (**Fig.** 1). Despite the potential uses of seaweeds their action on SMSs is not well explored. Our preliminary screening of Japanese dietary seaweed methanol extracts suggested that **wakame** is a promising candidate with strong inhibition specifically towards the SMS2 enzyme. Hence, this project was designed and proposed to find the bioactive component of **wakame** responsible for SMS inhibition which in turn helps in obesity control.


**Fig. 1** Research background and question.

２．研究の目的

The main purpose of the proposed research is to (a) Develop a sensitive method for SMS inhibition screening assay method using liquid chromatography/mass spectrometry (LC-MS) (b) Identify the active component of wakame responsible for SMS inhibition and characterize its chemical structure for further biological studies. Our preliminary studies showed that wakame methanol extract can inhibit the SMS1 and SMS2 >50% at 100 µg/mL concentration. The proposed study aims to (1) Develop LC-MS based SMS inhibition screening assay method (2) Perform solvent-fraction based screening to find the active sub-fraction (3) Identification and Characterization of the active compound in Wakame by LC-MS and NMR techniques (4) Perform the SMS assay for the active compound and determine IC50 values for SMS1 and

SMS2. These research findings will help to develop Wakame-based nutritional supplements or pharmaceuticals to control obesity and its disorders.

３．研究の方法

The research method involves mainly the preparation of wakame methanol extracts on a large scale and performing the liquid-liquid partitions using various solvents by using a separating funnel. The crude methanol extract was prepared from the dried wakame by stirring its powder in methanol for 24 h in 3 cycles. Later, liquid-liquid extraction was performed using hexane, methanol, ether, and ethyl acetate. The crude solvent extracts were dissolved in dimethyl sulfoxide at a concentration of 10 mg/mL and an SMS inhibition screening assay was performed. To perform the assay a targeted LC-MS analysis of C6-ceramide (substrate) and C6-sphinomyelin (product) was used and the cell lines were HeLa expressing SMS1 and SMS2 independently. The details about the SMS assay method were published in an international peer-reviewed journal (10.1039/d4an00304g, Analyst 2024). Further, silica gel column chromatography was used for the separation and isolation of active compounds from each fraction. Based on the sequential screening assay for each extract the active component fraction was identified and isolated. To characterize the active component high-resolution mass spectrometry and nuclear magnetic resonance were applied.

４．研究成果

The summary of the results was described as follows. At first, to develop a method for SMS inhibition screening assay using liquid chromatography/mass spectrometry (LC-MS), the HeLa cells expressing SMS1 and SMS2 were prepared and cultured in the laboratory. The cell lysates were prepared in 20 mM lysate buffer and were used for the SMS assay as an enzyme source. The known inhibitor ginkgolic acid was used as a positive control, and the unnatural substrates C6-ceramide and C6-sphingomyelin were used to monitor SMS activity by LC-MS. and the wakame extracts were screened for SMS inhibition using a successfully optimized LC-MS method. The results obtained from the extraction and liquid-liquid partition of the wakame extract are shown in **Fig. 2**. As consistent with our

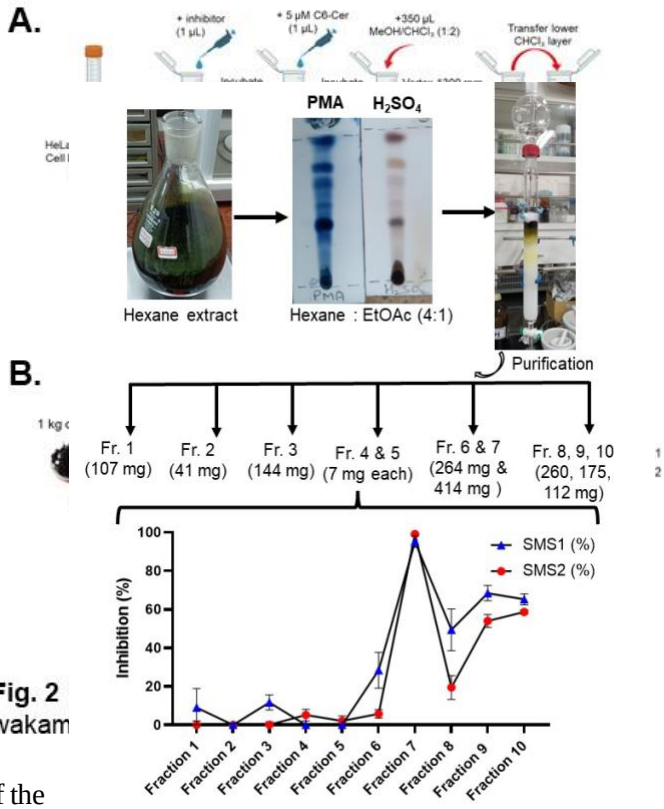


**Fig. 3** Hexane sub-fractions and their SMS inhibition results

**Fig. 2**
wakam

preliminary results hexane fraction showed the potential inhibition activity for SMS. Further, the hexane extract was sub-fractioned into 10 fractions using hexane: ethyl acetate (4:1) by column chromatography, and their SMS inhibition assay was conducted.

In that fraction 7 should have the highest inhibition for both SMS1 and SMS2. Additionally, fraction 7 was further fractionated into 4 fractions using hexane: ethyl acetate (4:1) and chloroform: methanol (9.5:0.5) in that fraction 7C showed the highest inhibition as shown in **Fig 3**. The active compound in fraction 7 was purified and characterized by HRMS and NMR techniques. As the results are unpublished the active compound is not revealed in this report. But it is named **WH73,** and SMS inhibition percentage was obtained for the active compound **WH73,** and the results are shown in **Table 1**.

**Table 1:** $IC_{50}$ of purified compound (SMS 1: 10 **µ**M , SMS2: 6.5 µM)

| Concentrations (µM) | SMS 1 Inhibition (%) | SMS 2 Inhibition (%) |
|---|---|---|
| 100 | 82 | 97 |
| 10 | 49 | 62 |
| 1 | -52 | -15 |
| 0.1 | -38 | -7 |

In summary, Wakame hexane extract showed the highest inhibition against both SMS1 and SMS2. Sub-fractionation of hexane extract was further screened for SMS inhibition and results showed that fraction 7 is active. Fraction 7 was further purified by chromatography techniques and characterized by HRMS and NMR techniques. The analysis confirmed that the active compound is a fatty acid **WH73** with potential SMS inhibitory activity. The purified compound showed $IC_{50}$ =10 µM for SMS1 and $IC_{50}$ = 6.5 µM SMS2 respectively. These results will be summarized for possible publication in an internal peer-reviewed journal. The possible relationship between the seaweed wakame and lipid metabolism associated with obesity was revealed in this research study.

５．主な発表論文等

〔雑誌論文〕　計14件（うち査読付論文　10件／うち国際共著　10件／うちオープンアクセス　1件）

| | |
|---|---|
| １．著者名<br>Nath Lipsa Rani、B. Gowda Siddabasave Gowda、Gowda Divyavani、Hou Fengjue、Chiba Hitoshi、Hui Shu Ping | ４．巻<br>447 |
| ２．論文標題<br>Dissecting new lipids and their composition in herbal tea using untargeted LC/MS | ５．発行年<br>2024年 |
| ３．雑誌名<br>Food Chemistry | ６．最初と最後の頁<br>138941～138941 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| １．著者名<br>Minami Yusuke、B. Gowda Siddabasave Gowda、Gowda Divyavani、Chiba Hitoshi、Hui Shu-Ping | ４．巻<br>184 |
| ２．論文標題<br>Regio-specific lipid fingerprinting of edible sea cucumbers using LC/MS | ５．発行年<br>2024年 |
| ３．雑誌名<br>Food Research International | ６．最初と最後の頁<br>114253～114253 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| １．著者名<br>Jayaprakash Jayashankar、B. Gowda Siddabasave Gowda、K. Shukla Pradeep、Gowda Divyavani、Nath Lipsa Rani、Chiba Hitoshi、Rao Radhakrishna、Hui Shu-Ping | ４．巻<br>9 |
| ２．論文標題<br>Sex-Specific Effect of Ethanol on Colon Content Lipidome in a Mice Model Using Nontargeted LC/MS | ５．発行年<br>2024年 |
| ３．雑誌名<br>ACS Omega | ６．最初と最後の頁<br>16044～16054 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| １．著者名<br>Gowda Siddabasave Gowda B.、Hou Fengjue、Gowda Divyavani、Chiba Hitoshi、Kawakami Kentaro、Fukiya Satoru、Yokota Atsushi、Hui Shu-Ping | ４．巻<br>1288 |
| ２．論文標題<br>Synthesis and quantification of short-chain fatty acid esters of hydroxy fatty acids in rat intestinal contents and fecal samples by LC-MS/MS | ５．発行年<br>2024年 |
| ３．雑誌名<br>Analytica Chimica Acta | ６．最初と最後の頁<br>342145～342145 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| 1．著者名<br>Malek Md Abdul、B. Gowda Siddabasave Gowda、Gowda Divyavani、Hui Shu-Ping | 4．巻<br>18 |
| 2．論文標題<br>Analysis of lipid composition and characterization of acyl steryl glycosides in rose petals by using nontargeted LC/MS | 5．発行年<br>2024年 |
| 3．雑誌名<br>Journal of Food Measurement and Characterization | 6．最初と最後の頁<br>2385～2396 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| 1．著者名<br>B. Gowda Siddabasave Gowda、Gowda Divyavani、Hou Fengjue、Shekhar Chandra、Chiba Hitoshi、Patzke Nina、Hui Shu-Ping | 4．巻<br>9 |
| 2．論文標題<br>Regiospecific analysis of lipidome in the brain from mammals of land and aquatic habitats-by liquid chromatography-mass spectrometry | 5．発行年<br>2023年 |
| 3．雑誌名<br>Heliyon | 6．最初と最後の頁<br>e22959～e22959 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| 1．著者名<br>Gowda Divyavani、Shekhar Chandra、B. Gowda Siddabasave Gowda、Chen Yifan、Hui Shu-Ping | 4．巻<br>3 |
| 2．論文標題<br>Crosstalk between Lipids and Non-Alcoholic Fatty Liver Disease | 5．発行年<br>2023年 |
| 3．雑誌名<br>Livers | 6．最初と最後の頁<br>687～708 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>該当する |

| | |
|---|---|
| 1．著者名<br>Gangadhara Rachana M.、Gowda Siddabasave Gowda B.、Gowda Divyavani、Inui Ken、Hui Shu-Ping | 4．巻<br>12 |
| 2．論文標題<br>Lipid Composition Analysis and Characterization of Acyl Sterol Glycosides in Adzuki and Soybean Cultivars by Non-Targeted LC-MS | 5．発行年<br>2023年 |
| 3．雑誌名<br>Foods | 6．最初と最後の頁<br>2784～2784 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| 1．著者名<br>B. Gowda Siddabasave Gowda、Shekhar Chandra、Gowda Divyavani、Chen Yifan、Chiba Hitoshi、Hui Shu‐Ping | 4．巻<br>No number |
| 2．論文標題<br>Mass spectrometric approaches in discovering lipid biomarkers for COVID‐19 by lipidomics:?Future challenges and perspectives | 5．発行年<br>2023年 |
| 3．雑誌名<br>Mass Spectrometry Reviews | 6．最初と最後の頁<br>No number |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

| | |
|---|---|
| 1．著者名<br>Gangadhara Rachana M.、Gowda Siddabasave Gowda B.、Gowda Divyavani、Inui Ken、Hui Shu-Ping | 4．巻<br>12 |
| 2．論文標題<br>Lipid Composition Analysis and Characterization of Acyl Sterol Glycosides in Adzuki and Soybean Cultivars by Non-Targeted LC-MS | 5．発行年<br>2023年 |
| 3．雑誌名<br>Foods | 6．最初と最後の頁<br>2784～2784 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.3390/foods12142784 | 査読の有無<br>無 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>－ |

| | |
|---|---|
| 1．著者名<br>B. Gowda Siddabasave Gowda、Gowda Divyavani、Hou Fengjue、Shekhar Chandra、Chiba Hitoshi、Patzke Nina、Hui Shu-Ping | 4．巻<br>9 |
| 2．論文標題<br>Regiospecific analysis of lipidome in the brain from mammals of land and aquatic habitats-by liquid chromatography-mass spectrometry | 5．発行年<br>2023年 |
| 3．雑誌名<br>Heliyon | 6．最初と最後の頁<br>e22959～e22959 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1016/j.heliyon.2023.e22959 | 査読の有無<br>無 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>－ |

| | |
|---|---|
| 1．著者名<br>Nath Lipsa Rani、B. Gowda Siddabasave Gowda、Gowda Divyavani、Hou Fengjue、Chiba Hitoshi、Hui Shu Ping | 4．巻<br>447 |
| 2．論文標題<br>Dissecting new lipids and their composition in herbal tea using untargeted LC/MS | 5．発行年<br>2024年 |
| 3．雑誌名<br>Food Chemistry | 6．最初と最後の頁<br>138941～138941 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1016/j.foodchem.2024.138941 | 査読の有無<br>無 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>－ |

| | |
|---|---|
| 1．著者名<br>Minami Yusuke、B. Gowda Siddabasave Gowda、Gowda Divyavani、Chiba Hitoshi、Hui Shu-Ping | 4．巻<br>184 |
| 2．論文標題<br>Regio-specific lipid fingerprinting of edible sea cucumbers using LC/MS | 5．発行年<br>2024年 |
| 3．雑誌名<br>Food Research International | 6．最初と最後の頁<br>114253～114253 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1016/j.foodres.2024.114253 | 査読の有無<br>無 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>－ |

| | |
|---|---|
| 1．著者名<br>Sundaraswamy Punith M.、Minami Yusuke、Jayaprakash Jayashankar、B. Gowda Siddabasave Gowda、Takatsu Hiroyuki、Gowda Divyavani、Shin Hye-Won、Hui Shu-Ping | 4．巻<br>No number |
| 2．論文標題<br>A facile method for monitoring sphingomyelin synthase activity in HeLa cells using liquid chromatography/mass spectrometry | 5．発行年<br>2024年 |
| 3．雑誌名<br>The Analyst | 6．最初と最後の頁<br>No number |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスではない、又はオープンアクセスが困難 | 国際共著<br>該当する |

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　1件）

| |
|---|
| 1．発表者名<br>Divyavani Gowda, Siddabasave Gowda B. Gowda,千葉仁志, 惠淑萍 |
| 2．発表標題<br>Detection of potential lipid biomarkers for acute kidney injury using LC/MS. |
| 3．学会等名<br>63rd Annual meeting of the Japanese Society of Clinical Chemistry (JSCC), Tokyo, Japan. |
| 4．発表年<br>2023年 |

| |
|---|
| 1．発表者名<br>Divyavani Gowda, Siddabasave Gowda B. Gowda, Atsuko Ikeda, Rahel Mesfin Ketema, Yu Ait Bamai, Reiko Kishi, Hitoshi Chiba, Shu-Ping Hui |
| 2．発表標題<br>Determination of plasma cholesteryl ester levels in preadolescent children by LC/MS |
| 3．学会等名<br>12th International Singapore Lipid Symposium（国際学会） |
| 4．発表年<br>2024年 |

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
|---|---|---|---|

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
|---|---|