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# *In vitro* EVALUATION OF ANTIPROLIFERATIVE AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACTS OF *Gracilaria corticata* AND *Gracilaria foliifera* AGAINST BREAST CANCER CELLS

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## ABSTRACT

**The present study was aimed to assess the methanolic extracts of *Gracilaria corticata* and *G. foliifera* for anti-proliferative and anti-oxidant potency against breast cancer cell line MDA-MB-231. The antitumor activity of *G. corticata* methanolic extract (GCME) and *G. foliifera* methanolic extract (GFME) against MDA-MB-231 cells was assessed by 3-(4,5-dimethylthiazol-2-cyl)-2,5-diphenyltetrazolium bromide (MTT) assay. The extracts decreased cell viability of MDA-MB-231 cells with an estimated half-maximal inhibitory concentration ($IC_{50}$) of 0.18 ± 1 and 0.078 ± 1 for GFMEs; and 0.116 ± 1 and 0.107 ± 1 for GCMEs. GCME significantly increased ROS level after 48 h of treatment and decreased glutathione (GSH) level ($p<0.01$) in a dose-and time-response manner. Intracellular ROS in MDA-MB-231 cells was determined by staining with fluorogenic agent 2′,7′-dichloro-dihydrofluorescein diacetate ($H_2$DCFDA). The mitochondrial membrane potential ($\Delta\psi_m$) disruption of GFME and GCME treated cells were maximum at 0.078 and 0.107 µg µL$^{-1}$ ($p<0.005$). *G. foliifera* showed significantly higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (78.6%) as compared to *G. corticata* (68.3%). This study also explored the phytochemical constituents of GFME and GCME such as hexadecanoic acid, methyl ester and cholesta-4,6-dien-3-ol, (3 beta), through GC-MS analysis.**

**Keywords*:*** Antioxidant, antitumor, glutathione, *Gracilaria* sp., mitochondrial membrane potential

## INTRODUCTION

Cancer is a severe extortion to human health worldwide, and chemotherapy is only the standard method for its treatment (Narasimhan *et al.*, 2013). Despite progress in biomedical research, there is urgent need to develop new anticancer drugs and therapies. The marine environment has been a fascinating source of the compounds with exceptional and inimitable chemical features on which the chemical synthesis and modelling of new drugs can be established with more specificity and efficacy for therapeutics (Alves *et al.,* 2018). Recently, attention has been given to the use of the naturally occurring algal compounds as immunopharmacologic chemicals. Rhodophyta, a class in red algae, contains sulphated galactans like agar, agarose, mannans, xylans, and carrageenan, which possess anticancer, antiviral, neuroprotective, and antioxidant properties (Souza *et al.*, 2018). Genus *Gracilaria* of this class produces diverse bioactive metabolites, which reportedly are immuno-modulator, antibacterial, antiviral, antiinflammatory, antioxidant, antidiabetic, and anticancerous

(Jiang *et al.,* 2014; Fitria, 2015; Kurniasari *et al.,* 2018). The cytotoxicity of methanolic extract of *G. tenuistipitata* (MGET) from Kouhu Beach, Taiwan, on Ca9-22 oral cancer cells has previously been reported (Yeh, 2012). Two enone fatty acids, i.e. (*E*)-10-oxooctadec-8-enoic acid and (*E*)-9-oxooctadec-10-enoic acid isolated from *G. verrucosa* inhibited the production of inflammatory markers nitric oxide, IL-6, and TNF-α (Lee *et al.,* 2009). Sulphated polysaccharides of *G. cornea* reduced neutrophil migration and inhibited edema, revealing its analgesic and anti-inflammatory activity (Coura *et al.,* 2012). The International Agency for Research on Cancer has released a global cancer observatory wherein lung, breast, and colon cancers are reported to be the top three cancers in terms of cancer incidence and mortality (Cancer, 2018). These three cancer types are believed to cause one-third of the cancer burden worldwide. Keeping this in mind, the present study was aimed to evaluate the *in vitro* antiproliferative and antioxidant activity of methanolic extracts of red alga *Gracilaria corticata* and *G. foliifera* against human breast cancer cell line (MDA-MB-231).

## MATERIALS AND METHODS

### *Sampling and extract preparation*

*Gracilaria corticata* (J. Agardh), and *G. foliifera* (Forsskal) Borgesen were sampled along the Dwarka and Veraval shoreline, Gujarat, India. Seaweeds were brought to the laboratory in plastic bags along with seawater to prevent evaporation. Fresh thalli were thoroughly cleaned to remove epiphytes and necrotic parts and rinsed with distilled water to remove impurities and excess salts; shade-dried (7-15 days), followed by crushing to a fine powder using a kitchen-type blender. Methanolic extracts of *G. corticata* (GCME) and *G. foliifera* (GFME) were prepared by homogenizing the powder with constant shaking for upto 72 h using methanol as a solvent. The extracts from three repeated soakings were pooled and filtered using Whatman filter paper No. 4 and the obtained filtrate was evaporated using a rotary evaporator. The residues (crude extracts) attained were dissolved in dimethylsulfoxide (DMSO) to make a final concentration of 100 µg $mL^{-1}$. The extract was stored at -20˚C till further use.

### *Cell line and chemicals*

MDA-MB-231breast cancer cell lines were purchased from the National Centre for Cell Science (NCCS), Pune (India). MDA-MB-231 cells were cultured on DMEM (Dulbecco's modified Eagle medium), high glucose (Gibco by Life Technologies, USA) supplemented with 10% FBS (HiMedia Laboratories) at 37˚C, 5% $CO_2$ and 95% relative humidity. Seed stocks were preserved in DMSO at -80˚C. All the chemicals and solvents used were of highest purity grade.

### *Antiproliferative assay*

Viability of triple-negative MDA-MB-231 breast cancer cells in response to 0.1, 0.2, 0.5, 1.0, and 1.5 µg $\mu L^{-1}$ of GCME and GFME was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The protocol was slightly modified from Bittner *et al.* (2021). In brief, MDA-MB-231 cells were seeded in 96 well culture plates supplemented with 10% FBS at a density of 10,000 cells per well for 24 and 48 h. After completion of the desired growth period, cells were starved and later replenished with complete DMEM along with the GCME and GFME treatment in triplicates. Control cells were treated with DMSO. After 24 and 48 h, the cells were washed with 1X PBS and incubated with MTT reagent for upto 4 h in dark, thereafter the supernatant was discarded and formazan crystals formed were dissolved in 100 µL DMSO with constant shaking. Absorbance was recorded at 570 nm on BioTek™ Synergy™ H1 microplate reader against blank. Percentage of cell proliferation was calculated as 100% viable cells, wherein:

$$\% \text{ Viability} = \text{Mean OD in treated cells/ Mean OD in control cells} \times 100$$

***Measurement of intracellular reactive oxygen species***

**2',7'-dichlorodihydrofluorescein diacetate ($H_2$DCFDA) assay:** Total ROS was measured by using $H_2$DCFDA assay. Cells were seeded overnight in a 96-well plate at a density of 10,000 cells well$^{-1}$ and given applicable treatments. Then, the cells were washed with 1X PBS and incubated in dark for 30 min after equilibration with 10 µM $H_2$DCFDA. After washing twice with PBS, the fluorescence intensity was read at wavelength of 485 nm and emission wavelength of 535 nm using a microplate reader as per the modified protocol of Gill *et al.* (2017).

**Dihydroethidium (DHE) assay:** The changes in ROS levels within MDA-MB-231 cells were monitored after 0.18, and 0.078 µg µL$^{-1}$ GFME and 0.116 and 0.107 µg µL$^{-1}$ GCME treatments for 24 and 48 h, respectively. The cells were washed twice with 1X PBS and the fluorescence was measured using a microplate reader (BioTek™ Synergy™ H1), after staining with 10 µM DHE at 535/635 nm (excitation/ emission) (Mehra *et al.,* 2019).

***Mitochondrial membrane potential ($\Delta\psi_m$) analysis (JC-1 assay)***

The 5,5'6,6'-tetraethylbenzimidazolylcarbocyanine iodine (JC-I) probe was used to determine the changes in mitochondrial membrane potential (MTP). MDA-MB-231 cells were grown in 96 well culture plates at a density of 10,000 cells per well. Cells were treated with respective sublethal doses of GCME and GFME for 24 and 48 h at 37˚C. After serum starvation cells were washed with 1X PBS. Later on, stained by 20 µM JC-1 for 30 min at 37˚C. Fluorescence was recorded as a ratio of JC-1 monomer/aggregate at 485/535 and 550/600 nm using BioTek™ Synergy™ H1 microplate reader (Silva *et al.,* 2018).

***Measurement of total glutathione content***

Total GSH in the samples was determined by using Ellman's reagent (DTNB), which was based on the reaction between DTNB and GSH producing a yellow-coloured product. Proteins were precipitated with 25% trichloroacetic acid (TCA), followed by centrifugation at 6000 rpm for 10 min. The supernatant obtained was then mixed with DTNB and incubated for 15 min in dark. Absorbance was measured for 3 min at 412 nm using a BioTek microplate reader (Gill *et al.,* 2017). The GSH content was determined from a standard curve made using GSH.

***1, 1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity***

The free radical scavenging activity was measured by using DPPH (1,1-diphenyl-2-pikryl-hydrazin) solution in methanol. The decline in absorbance was recorded at 515 nm against a methanol blank over a period of 20 min (Yuan *et al.,* 2017). The radical scavenging activity (RSA) was calculated as follows:

$$\text{RSA (\%)} = (1 - \text{absorbance of sample/absorbance of control}) \times 100$$

***Gas chromatography-mass spectrometry (GC–MS) analysis***

The methanolic extracts of *G. corticata* and *G. foliifera* were analysed by GC-MS using Shimadzu QP 2010 Ultra, DB-5, a 30-m fused silica capillary column (0.2 mm, ID, 0.25 µm film thickness) with helium as a carrier gas (1.0 mL min$^{-1}$). The column temperature was programmed from 60°C with 2 min hold time followed by a ramping rate at 25°C min$^{-1}$ upto 150°C min$^{-1}$ and then from 10°C min$^{-1}$ upto 300°C min$^{-1}$. The sample was injected in spitless mode. Data for quantitative analysis was acquired in the electron impact (EI) mode (70 eV) with ion source maintained at 200°C for 10 min and scan-interval of 0.50 sec. The compounds were identified by comparison of their mass spectra with those of WILEY8.LIB and National Institute of Standards Technology (NIST) MS library in (NIST11s.lib) (Konda *et al.,* 2017). The names and molecular weights of the components of tested solvents were ascertained.

***Statistical analysis***

Data analysis was performed by using a one-way analysis of variance test by GraphPad Prism 6 for Windows (GraphPad Software San Diego, USA). Data were presented as mean ± standard deviation (n = 3). P-value of *p<0.001, **p<0.005 and ***p <0.001 were considered statistically significant.

## RESULTS AND DISCUSSION

Cancer has become a major threatening problem in today's world and the cancer patients need special treatment and critical care. The progress in the field of natural anticancer drug development allows the advancement towards better therapeutic efficacy. Recently, red marine algae (Rhodophyta) have gained significant attention because of their wide range of biological activities and immense nutraceutical and therapeutically benefits. The cytotoxicity of GCMEs and GFMEs was tested on MDA-MB-231 cell lines by MTT assay. The maximum potency against MDA-MB-231 cells was observed after 48 h of treatment, with cell viability of 12.05% ($IC_{50}$ 0.078 ± 1) for GFMEs and 20.41% ($IC_{50}$ 0.116 ± 1) for GCMEs (Fig. 1). The cytotoxic effect of *G. foliifera* and *G. corticata* observed were in agreement with Erfani *et al.* (2015) and Jenifer *et al.* (2017). In present study *G. foliifera* (93.4%) exhibited the highest toxic effect on MDA-MB-231 cells, followed by *G. corticata* (84.3%). Narasimhan *et al.* (2013) reported the toxicity of *G. corticata* (93.75%) on MCF-7 cells along with 79% ROS scavenging activity.

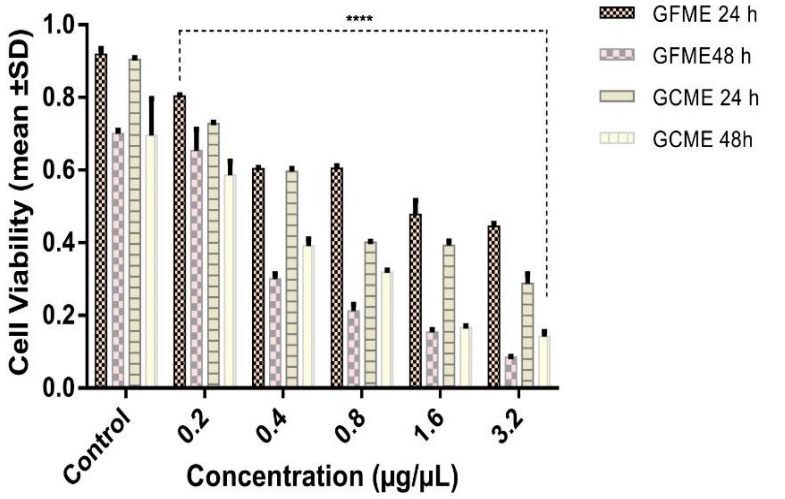


**Fig. 1: Proliferation of MDA-MB-231 breast cancer cells is inhibited by GFME and GCME. Data are expressed as mean ± SD (n = 3). The cell viability of treated MDA-MB-231 breast cancer cells significantly decreased in a dose-response manner (p< 0.001).**

DHE and $H_2$DCFDA assays revealed the intracellular ROS levels in GFME and GCME treated MDA-MB-231 cell lines (Fig. 2). A significant increase in intracellular ROS was observed, which as compared to the untreated control cells after 48 h of treatment with sublethal doses of GFMEs and GCMEs was 1.5-3.5 folds in DHE (Fig. 2A) and approximately 1.5-fold in $H_2$DCFDA (Fig. 2B). GCME showed the strongest intracellular ROS scavenging effects at $IC_{50}$ of 0.107 µg $\mu L^{-1}$ after 48 h of treatment.

The fluorescence intensity in DHE and DCF-positive treated cells was significantly high as compared to the respective untreated MDA-MB-231 cells. The increased green fluorescence in case of GCME ($IC_{50}$ of 0.107 µg $\mu L^{-1}$) after 48 h (Fig. 2A) of treatment, represents the beginning of apoptosis in MDA-MB-231 cells. This observation is in line with Acharya *et al.* (2020).

The loss of $\Delta\psi_m$ in treated cells was confirmed by increase in fluorescence intensity after labelling with dye JC-1. In untreated control cells, JC-1 formed oligomeric mitochondrial aggregates, which emitted strong red fluorescence; whereas, untreated cells JC-1 did neither form any aggregate nor remained as a monomer emitting green fluorescence. Treated cells showed decrease in mitochondrial red fluorescence and increase in green fluorescence, suggesting that the treatment disrupted the mitochondrial $\Delta\psi_m$. A significant time-dependent increase in MMP (1.5-3.5-fold) was observed, with maximum MMP disruption at 0.078 and 0.107 µg $\mu L^{-1}$ concentration of GFMEs and GCMEs, respectively, after 48 h of treatment (Fig. 3A).

After treatment with GCME and GFME for 48 h, the MDA-MB-231 cells showed decrease in glutathione (GSH) level (Fig. 3B). A reduction in GSH, a known redox buffer of cell with chemoprotective action, results in increase in ROS production which causes apoptosis. The decrease in GSH was statistically significant ($p < 0.01$) at the concentrations of 0.078 and 0.107 µg

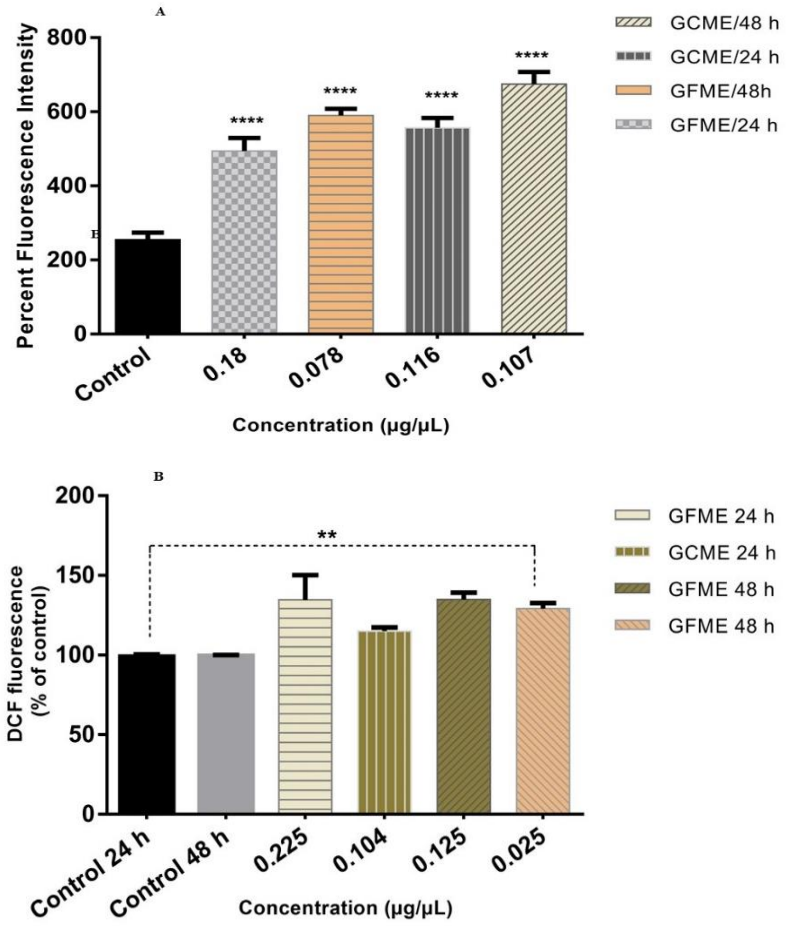


**Fig. 2: Intracellular ROS levels in MDA-MB-231 cells are determined by DHE (A) and $H_2$DCFDA (B). Results are expressed in terms of mean percent DHE fluorescence intensity in comparison to control. The data represent the mean values ± SD (n = 3); **p < 0.005, ****p < 0.001).**

$\mu L^{-1}$ as compared to the control cells. In cells the ratio of reduced glutathione (GSH): oxidized glutathione (GSSG) is maintained at optimum level and this ratio is critical for cell survival. Decrease in reduced form of glutathione may lead to the oxidative damage within the cells (Patra, 2013). GSH plays vital role in cell protection against intra-cellular ROS, which induces apoptosis. Depletion in GSH level increases ROS susceptibility, and ultimately damages the cell (Kang *et al.*, 2012). The ROS level in MDA-MB-231 cells showed gradual increase after GFME and GCME treat-ment for 48 h, suggesting that both the extracts might induce apoptosis by depleting intracellular GSH and increasing intracellular ROS.

Antioxidant activity of methanolic extracts of *G. foliifera* (GFME) (78.61% quenching) and *G. corticata* (GCME) (68.35% quenching) was determined by DPPH radical scavenging activity, which was more than 65% scavenging for DPPH radical (100 µM), as compared to the standard antioxidant ascorbic acid (Fig. 4).

Along with GSH depletion, mitochondria play an important role in ROS and apoptotic events (Silva *et al.*, 2018; Batista *et al.*, 2020). Yeh *et al.* (2012) reported that the methanolic extracts of *G. tenuistipitata* (MEGT) significantly decreased the intensity for mitochondrial membrane potential (MMP) of MEGT-treated Ca9-22 cancer cells in a dose-dependent manner ($p < 0.05$). Pieme *et al.* (2014) also reported that natural products could disrupt mitochondrial potential inducing apoptosis in cancer cells. Our results are in agreement with them which demonstrated the dramatic MMP disruption in breast cancer cells in a time and response-dependent manner, thereby signifying that the ability of GFME and GCME to induce MMP disruption might directly or indirectly interfere ROS generation in breast cancer cells. However, MMP disruption was maximum in GCME after 48 h of treatment as compared to GFME, which may result in the release of cytochrome coenzyme and initiate the apoptotic events (Acharya *et al.*, 2020).

During GC-MS analysis of GCME and GFME, various active constituents with strong antioxidant activity were observed. Table 1 depicts the presence of 2,4-ditert-butyl-phenol; 2,6,10-trimethyl,14-ethylene-14-pentadecne; hexadecanoic acid, methyl ester; 2-hexadecen-1-ol, 3,7,11,15-

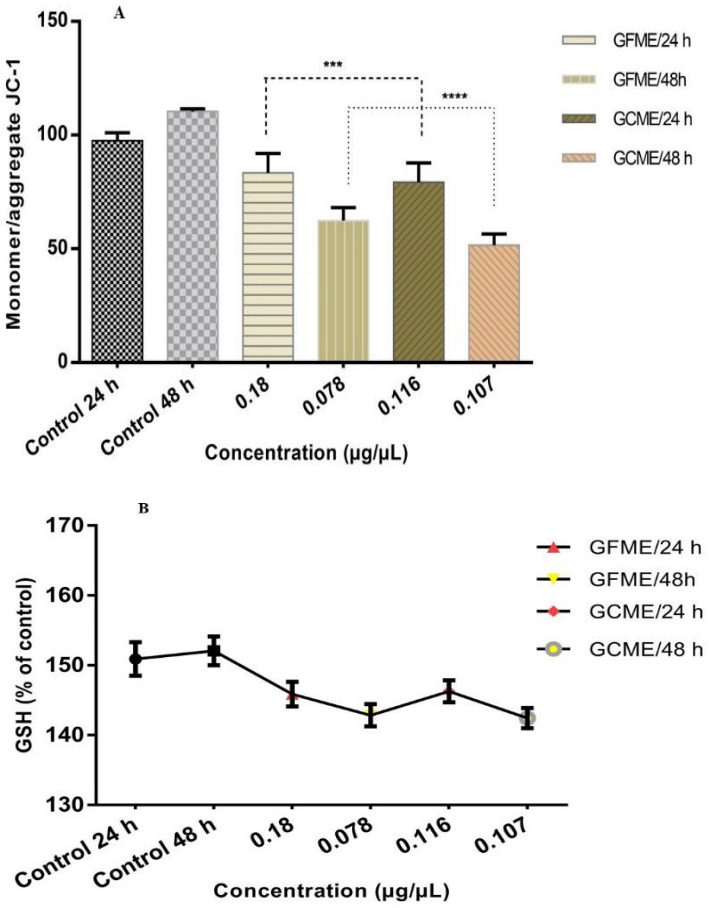


**Fig. 3: A) Dose and time-dependent mitochondrial membrane hyperpolarization is depicted by GCME and GFME. Results are expressed as mean monomer/aggregate JC-1 ratio (**p < 0.005). B) Measurement of total glutathione (GSH) levels in GFME and GCME treated cancer cells with respect to untreated control cells. Data was statistically significant at *p<0.01, results are presented as mean ± SD (*n* = 3)**

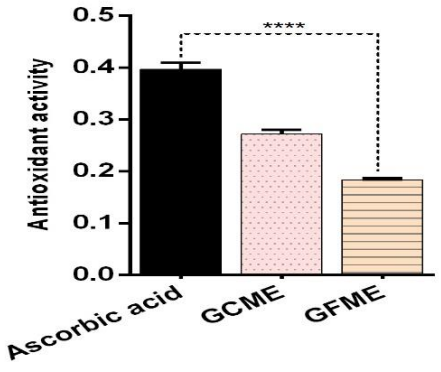


**Fig. 4: DPPH radical scavenging of methanolic extracts of *Gracilaria foliifera* and *G. corticata,* and ascorbic acid**

tetramethyl-[R-[R*, R*-(E)]]; cholesta-4,6-dien-3-ol, (3 beta); etc. from GCME. Whereas, dodecyl acrylate; 2,6,10-trimethyl, 14-ethylene; 1,16-hexa-decanediol; 9-octadecenamide; 1,2-benzene dicarboxylic acid, etc. were reported from GFME. These constituents possess diverse biological activities *viz.*, antioxidant, antiinflammatory, anti-tumor, antimicrobial, chemo-preventive, and antiprotozoal activities. Similar results have been reported from genus *Gracilaria* by Arulkumar *et al.* (2018) who confirmed the presence of eugenol, pentatriacontane, sulfurous acid, 2-ethylhexyl isohexyl ester and phthalic acid phytochemicals, highlighting that the total phenol content ($4.00 \pm 0.35$ mg GAE $g^{-1}$) was responsible for the antioxidant potential and DPPH radical scavenging activity (23.95%) of *G. corticata*. Gunathilaka *et al.* (2019) and Rajivgandhi *et al.* (2020) reported that phenol and flavonoid were responsible for the excellent antioxidant activity of *G. corticata* and *G. foliifera*. Lee and Kim (2015) evaluated the antioxidant activity of 51 marine algae including *G. verrucosa* (30.41 ± 2.88) and other Rhodophyta collected from Korea. Thus, higher antioxidant and antiproliferative activity observed in GFME and GCME might be due to the presence of these phytochemicals (Table 1). Jenifer *et al.* (2017) demonstrated that the methanolic extract of *G. foliifera* collected from Manapad coast, Tamil Nadu, India had potent antioxidant activity ($IC_{50}$ of 679.27 µg $mL^{-1}$). They also confirmed the presence of 1,2-benzene dicarboxylic acid in *G. corticata*, which is cytotoxic against MCF-7 breast cancer cell lines with $IC_{50}$ of 100 µg $mL^{-1}$ and 78.14% cell inhibitory effect (Krishnan *et al.*, 2014; Jenifer *et al.*, 2018). These results are in line with our findings of 68.35 and 78.61% of DPPH radical scavenging activity by GFME and GCME, respectively.

**Table 1: Phytochemicals identified through GC-MS analysis in the methanolic extracts of *Gracilaria corticata* and *G. foliifera***

| Retention time | Name | Molecular formula | Mol. wt. | Area (%) | Nature of compound | Reported activity |
|---|---|---|---|---|---|---|
| | | ***Gracilaria corticata* methanol extract** | | | | |
| 9.865 | 1-Dodecene | $C_{12}H_{24}$ | 168 | 0.49 | Alkene | Antioxidant (Nimbeshaho *et al.*, 2020) |
| 14.520 | 2,4-Ditert-butylphenol | $C_{14}H_{22}O$ | 206 | 4.47 | Phenol | Antioxidant, anti-cancer, anti-fungal and antibacterial activity (Hajjar *et al.*, 2017) |
| 18.500 | 2,6,10-Trimethyl,14-ethylene-14-pentadecne | $C_{20}H_{38}$ | 278 | | Olefins | Antiproliferative (Selvamangai Bhaskar, 2012) |
| 19.451 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 7.55 | Methyl palmitate | Antioxidant, anti-inflammatory (Hamed *et al.*, 2020) |
| 19.567 | Benzenepropanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, methyl ester | $C_{18}H_{28}O_3$ | 292 | 0.61 | - | - |
| 21.380 | 2-Hexadecen-1-ol, 3,7,11,15 -tetramethyl-, [R-[R*, R*-(E)]] | $C_{20}H_{40}O$ | 296 | 1.57 | Phytol (Diterpene) | Antioxidant, antinociceptive (Santos *et al.*, 2013) |
| 21.499 | Methyl stearate | $C_{19}H_{38}O_2$ | 298 | 0.97 | Fatty acid | Antioxidant, anti-bacterial (Hagr Adam, 2020) |
| 28.519 | Cholesta-4,6-dien-3-ol, (3 beta) | $C_{27}H_{44}O$ | 384 | 1.72 | Steroid | Antioxidant, antiviral, anti-fungal, and anti-bacterial (Cotas *et al.*, 2020)] |
| | | ***Gracilaria foliifera* methanol extract** | | | | |
| 14.510 | Phenol, 2,4-bis(1,1-dimethylethyl) | $C_{14}H_{22}O$ | 206 | 1.27 | Phenol | Antioxidant (María Teresa *et al.*, 2014; Padmavathi *et al.*, 2014) |
| 16.820 | Dodecyl acrylate | $C_{15}H_{28}O_2$ | 240 | 0.65 | Carboxylic acid | Higher radical scavenging activities (Fahem *et al.*, 2020) |
| 18.500 | 2,6,10-trimethyl,14-ethylene | $C_{20}H_{38}$ | 278 | 1.01 | Olefins | Antiproliferative (Selvamangai Bhaskar, 2012) |
| 19.440 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 3.57 | Palmitic acid | Antioxidant, anti-inflammatory, hypocholesterolemic, nematicide, 5-alpha reductase inhibitor (Muflihunna *et al.*, 2021) |
| 20.485 | 1,16-Hexadecanediol | $C_{16}H_{34}O_2$ | 258 | 0.47 | Ester | Cytotoxic (Ren *et al.*, 2014) |
| 21.370 | 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]- | $C_{20}H_{40}O$ | 296 | 2.83 | Phytol, | Anti-cancer, antioxidant, anti-inflammatory, diuretic, cytotoxicity, and anti-microbial (Kim *et al.*, 2020) |
| 23.440 | Dotriacontane | $C_{32}H_{66}$ | 450 | 1.14 | Alkanes | Antioxidant (Asong *et al.*, 2019) |
| 25.180 | 1,2-benzenedicarboxylic acid | $C_{24}H_{38}O_4$ | 390 | 1.43 | Phthalic acid | Antioxidant and antimicrobial (Rubab, 2018) |
| 26.480 | Hexacosane | $C_{26}H_{54}$ | 366 | 0.80 | Organic acid (alkane) | Antioxidant (Elshamy *et al.*, 2019) |
| 27.270 | 9-Octadecenamide | $C_{18}H_{35}NO$ | 281 | 3.72 | oleic acid | Antioxidant and antimicrobial (Kim *et al.*, 2020) |
| 28.310 | Tetratetracontane | $C_{43}H_{88}$ | 604 | 0.28 | Alkane | Antioxidant, and cytoprotective activities (Kawuri Darmayasa, 2019) |
| 31.508 | Cholest-5-en-3-ol (3 beta)- | $C_{27}H_{46}O$ | 386 | 8.94 | Cholesterol | Anticancer activity (Khan, 2019) |

**Conclusions:** The MTT assay, DHE and $H_2$DCFDA staining, GSH depletion, JC-1 assay, and DPPH assay revealed that GCME and GFME act as possible candidates for further fractionation to

develop a novel anticancer drug. These preliminary studies suggest that *Gracilaria* species have a wide range of phytochemical constituents that may be the new source of natural products with potential uses in nutraceutical, pharmacological and cosmetic industries for their antioxidative and antiproliferative effects. The present information generated can be used as an alternative method for the treatment of cancer apart from chemotherapy and surgery. However, further *in vitro* and *in vivo* anti-cancer investigations are required to search for novel anticancer drugs.

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**Author contributions statement:** A.R.: Study design, sampling, experimental work, data analysis, writing, drafting the manuscript. D.S.Y. and A.K.: assisted during experiments. V.J.: guiding, editing and reviewing. FB: guidance, proof checked and finalized the manuscript. All the authors reviewed the manuscript.

**Conflict of interest:** All the authors declare that they have no capitating interest.

**Ethical statement:** This study does not involve any active human participants as the openly accessible datasets have been utilized.

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