Cytotoxic effect of acetogenins and sesquiterpenes obtained from the Red alga Laurencia majuscula

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Abstract

Purpose: To evaluate the cytotoxicity of n-hexane extract and its metabolites obtained from the red alga, Laurencia majuscula, against three cancer cell lines HCT-116 (colon cancer), PC-3 (prostate cancer) and HepG2 (liver cancer) cells; and to identify the phytochemical compound(s) involved.

Methods: Solvent extraction, thin layer chromatography, aluminum oxide column chromatography, and preparative thin layer chromatography (PTLC) were employed for isolating pure compounds from n-hexane extract of Laurencia majuscula. Nuclear magnetic resonance (NMR) and mass spectrometry (MS) measurements were used for structural elucidation of the compounds. The cytotoxicity of the non-polar extract and isolated compounds were evaluated against HCT, PC-3, and HepG2 cells using MTT assay, relative to the standard cytotoxic drug (cisplatin).

Results: Three sesquiterpenes (1, 2 and 8), and five acetogenins (3-7) were isolated from the n-hexane extract. The n-hexane extract showed higher potent cytotoxic effect than sesquiterpenes and the acetogenins (3-7).

Conclusion: These results indicate that the n-hexane extract of Laurencia majuscula exerts significant cytotoxicity against HCT-116, PC-3 and HepG2 cell lines, thus suggesting that the plant extract may be effective chemotherapeutic agents for the management of colon, prostate and liver cancer.

Keywords: Red Sea alga, Rhodomelaceae, Polyketides, Terpenes, Anticancer

INTRODUCTION

Marine red algae comprise of diverse bioactive compounds that exert antimicrobial, anti-inflammatory, cytotoxic, antifoulants, insecticidal and immunosuppressive effects [1,2]. It has been reported that the genus Laurencia is the most productive in the Rhodomelaceae genera. Sesquiterpenoids, diterpenoids, and cyclic and polycyclic haloethers (C15 acetogenins) are polyketides. Steroids are frequently isolated from Laurencia species [1-3]. The diversity of halogenated metabolites makes them play vital roles as chemotaxonomic markers [4-6]. However, not much is known about the bioactive constituents of Laurencia majuscula specifically responsible for its activity. Thus, the present study was carried out to investigate the cytotoxicity of n-hexane extract of Laurencia majuscula, and to isolate and identify the
cytotoxic compounds in their pure forms.

EXPERIMENTAL

Materials

Laurencia majuscula Lamouroux was collected in September, 2018 from Al-rays area, Saudi Arabia (23° 34’ 11.3” N; 38° 36’ 10.6” E). A voucher sample (JAD 0366) was deposited at Faculty of Marine Science, King Abdulaziz University.

Cell lines and reagents

The three cell lines HCT-116 (colon cancer), PC-3 (prostate cancer), and HepG2 (liver cancer) were purchased from American Type Culture Collection. All cells were cultured in DMEM (12-604F, Lonza Verviers SPRL, Belgium) supplemented with 5 % fetal bovine serum (S-001B-BR, Life Science Group L, UK); 100 IU/mL penicillin and 100 µg/mL streptomycin (17-602E, Lonza Verviers SPRL, Belgium). The rest of assay was performed as previously reported [7,8].

Extraction and isolation

Laurencia majuscula (260 g) was dried, and extracted with n-hexane. The n-hexane extract (4.2 g) was fractionated on a neutral aluminum oxide column, employing gradient elution (n-hexane: diethyl ether), and 50-ml fractions were collected. The fractions were combined into four pools (A, B, C and D). Pool A, which was eluted with n-hexane: diethyl ether (98:2, v:v), was purified on a neutral aluminum oxide column, and it yielded a fraction containing compounds 1 and 2. Compounds 1 and 2 were purified using Si-Gel PTLC and eluted with n-hexane. Pool B, which was eluted with n-hexane: ether (95:5, v:v) was purified on Si-Gel PTLC and eluted with n-hexane/diethyl ether mixture to yield compounds 3-5. Fraction C, which was eluted with n-hexane: ether (85:15, v:v) was purified on Si-Gel PTLC and eluted with n-hexane/diethyl ether (85:15, v:v) to yield compounds 6 and 7. Pool D, which was eluted with n-hexane: diethyl ether (80:20, v:v) was purified on Si-Gel PTLC. Elution was done with n-hexane: diethyl ether (75:25 v:v) to yield compound 8.

Cytotoxicity assay

The cancer cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for 24 h at 37°C in an incubator containing 5% CO2. Thereafter, the cells were treated with serial dilution of the Conus extract (50, 25, 12.5, 6.25, 3.125, and 1.56 µg/mL) and after 48 h, the viability of each cancer cell line was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 5 mg/mL) assay which measures the activity of mitochondrial succinate dehydrogenase in viable cells. The cells were incubated for another 4 h and the formazan crystals were solubilized using 10 % SDS/PBS/0.01 N HCl. After 14 h, the absorbance of the formazan solution was measured at 570 nm and A 630 nm using BioTek plate reader (EL x 808, BioTek Instruments, Inc., Winooski, VT, USA). The assay was carried out in triplicate. The IC50 was calculated in terms of the concentration that caused 50 % inhibition of cell growth [8].

Statistical analysis

All statistical analyses were performed using GraphPad InStat software, version 3.05 (GraphPad Software, La Jolla, CA). Graphs were plotted using GraphPad Prism software, version 6.00 (GraphPad Software, La Jolla, CA).

RESULTS

Extensive fractionation of the n-hexane extract of Laurencia majuscula, employing different chromatographic techniques, led to isolation of eight metabolites (1-8) namely: 4,10-dibromo-3-chloro-7(14)-chamigrene (1); α-isobromocuparene (2); (12E)-cis-maneonene A (3); (12Z)-cis-maneonene D (4); (12E)-cis-maneonene E (5); bromlaurenidifin (6); jeddahenyne A (7) and cuparen-3-ol (8). The structures of these compounds were elucidated via interpretation of their spectra, including 1D and 2D as well as NMR and MS, and comparison with reported data [9-14].

The cytotoxicity of the non-polar extract and isolated compounds 1-8 (Figure 1) were assessed against three cancer cell lines HCT-116, PC-3 and HepG2, with cisplatin as standard cytotoxic drug, using MTT assay. The results are shown in Table 1.

DISCUSSION

The genus Laurencia (Rhodomelaceae) comprises 146 taxonomical species, and it is recognized as one of the best sources of promising secondary metabolites. The worldwide distribution (tropical, subtropical, and temperate coastal waters) and chemical diversity of these species are due to genetic and environmental factors which have resulted in a limitless array of natural compounds [9].
Cancer is one of the most serious illnesses in humans, and one of the leading causes of death worldwide [15]. Natural compounds with potent biological effects are considered as lead and promising reservoirs from which modern medicine is derived, particularly for treatment of cancer. In vitro cell proliferation assays are most widely used for evaluating preliminary antitumor effects of synthetic and natural compounds. These tests give an indication of cytotoxicity, but in order to determine the mechanism of action, specialized assays are required [16]. On these bases, Laurencia majuscula extract was fractionated to eight compounds which were evaluated for their cytotoxic effects against three cancer cell lines.

The extract showed significant cytotoxic effect against the three cell lines tested: HCT-116 (human colon cancer cells), PC-3 (human prostate cancer cells) and HepG2 (human hepatocellular carcinoma cells). The extract, isolated compounds and cisplatin (positive control) showed significant cytotoxic activities against HCT-116, PC-3 and PC-3. Based on these data, it is obvious that the n-hexane extract was more potent than the isolated compounds. This could be due to some sort of synergistic/agonistic effect of the compounds, or it could be that the bioactive compound in its natural form is more potent than the isolated one.

**CONCLUSION**

The n-hexane extract of the red alga, Laurencia majuscula, exhibits cytotoxic effect against three cancer cell lines HCT-116, PC-3 and HepG2. Fractionation of the extract led the isolation of eight terpenoid derivatives. The isolated sesquiterpenes are more potent than the isolated acetogenins containing polyketides.

**DECLARATIONS**

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

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