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Isolation of Hopenone-I from the Leaves of Mangrove Plant *Scyphiphora hydrophyllacea* and Its Cytotoxic Properties

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SRS, KHT, EDS and MKE were involved for the designing of the study. Authors SRS, NF, MKE and LW were involved in conducting the experiments, writing the manuscript and analyzing data. Author AA was involved for structure elucidation. Manuscript corrections were done by authors SRS, AA and KHT. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The present study was aimed to isolate active anti proliferative compound/s from the hexane extract of leaves of the mangrove plant *Scyphiphora hydrophyllacea* C.F.Gaertn. and evaluate their cytotoxic properties.
Place and Duration of the Study: At the Institute of Biochemistry, Molecular Biology and Biotechnology and HEJ Research Institute of Chemistry, between January 2015 to November 2015.
Methodology: The hexane extract of the leaves of *S. hydrophyllacea* which was found to be cytotoxic was fractionated using normal phase column chromatography and fractions obtained from

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column chromatography were tested for anti proliferative effects. Active fractions obtained were further fractionated using normal phase column chromatography to obtain compound **1**. Structure of the isolated compound was elucidated with combination of ^{1}H- and ^{13}C-NMR spectroscopy, and mass spectrometry techniques. Anti proliferative properties of compound **1** in three cancer cell lines {MCF-7 (estrogen receptor positive breast cancer), HepG2 (hepatocellular carcinoma) and AN3CA (endometrial cancer)} was evaluated by MTT assay after 24 and 48 h incubations.

Results: Isolated compound was found to be hopenone-I, a triterpenoid and this is a novel finding for the presence this compound in the mangrove plant *S. hydrophyllacea.* Hopenone-I showed some promising anti proliferative effects in all the cancer cells in a time and dose dependent manner after 24 and 48 h incubations {MCF-7: 14.98 µM (24 h) and 7.76 µM (48 h), HepG2: 29.24 µM (24 h) and 11.60 µM (48 h), AN3CA: 12.15 µM (24 h) and 4.99 µM (48 h)}.

Conclusion: Based on the overall results of the present study, we conclude that hopenone-I isolated for the first time from *S. hydrophyllacea* leaves possess prominent anti proliferative effects in MCF-7, AN3CA and HepG2 cell lines.

Keywords: Scyphiphora hydrophyllacea; hopenone-I; mangrove; cytotoxic effects.

1. INTRODUCTION

Cancer is considered as the most common cause of mortality and morbidity in the world [1]. Despite recent advanced treatment options for cancer, possible clinical outcomes are still beyond the expectations and have not improved over the last few decades [2]. Standard treatment options for cancer include radiotherapy, chemotherapy, hormone therapy and surgery [3]. However, chemotherapy and radiotherapy will result severe side effects to the patient. Chemotherapy and radiotherapy will be the only available treatment when surgical removal would cause some functional loss in the cancer patient. Therefore it is essential to search for new alternative treatment for chemotherapy with fewer side effects. Plant derived natural compounds play a pivotal role in the development of cancer drugs and several plant derived natural compounds have been launched in clinical trials [4]. Mangrove plants have been used in traditional medicine to treat various diseases including cancers in the world and several natural compounds are reported to be isolated from mangrove eco system [5]. *S. hydrophyllacea* (Family: Rubiaceae), a mangrove plants, is found in Asia, Caroline Islands, Australia, and New Caledonia [6]. Previously researchers have found that flavonoids and terpenoids, especially iridoids, are major constituents of *S. hydrophyllacea* [7]. Previous studies have revealed that this plant mainly contains known flavones and triterpenoids, among which betulone shows anti proliferative effects on human hepatoma SMMC-7721 cell line [8]. The present study was designed to isolate active anti proliferative compound in the active hexane extract of *S. hydrophyllacea* leaves and to evaluate its anti-proliferative properties in three different cancer cell lines (HepG 2, MCF-7 and AN3CA).

2. METHODOLOGY

2.1 General

The NMR Spectra were measured in $CDCl_3$ on a Bruker AV-500 NMR instrument (Fällanden, Switzerland). EIMS was recorded on electron impact mode on Finnigan MAT-312 and MAT-95 XP spectrometers, and ions are given in m/z (%). Silica gel 60 (230-400 mesh) was purchased from Merck, Darmstadt, Germany. Pre-coated silica gel plates (Merck, Kiesel gel 60 F254, 0.25 mm) were used for thin layer chromatography studies. All the cell lines and cell culture reagents were purchased from American Type Culture Collection (ATCC, Manassas, USA) and all the chemical in the study were purchased from Sigma-Aldrich (St Louis, MO, USA) unless otherwise specified.

2.2 Collection of Plant Material

Leaves of *S. hydrophyllacea* were collected from National Aquatic Resources Research and Development Agency (NARA) Negombo, Sri Lanka and plant material was identified by Mr W.A. Sumanadasa, Research Officer at NARA. Voucher specimen was deposited (Voucher specimen: S-11) at the Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo Sri Lanka. Collected plant material was washed, dried and ground. Ground material was kept in the freezer until used for further experiments.

2.3 Extraction and Isolation

Dried plant material (200 g) was extracted into hexane (500 mL x 3 times) by sonicating for 1 h at room temperature. Since our preliminary research indicated that the hexane extract of leaves of *S. hydrophyllacea* possess anti proliferative properties (Unpublished data), here we selected the hexane extract to isolate possible cytotoxic compounds. Resulting extracts were filtered and evaporated under reduced pressure at -20°C to obtain 4.07 g of dry weight. Then the resulting hexane extract (4 g) was separated in a silica gel column with hexane-ethyl acetate (100: 0 - 0: 100 v/v), methanol-ethyl acetate (20: 80 v/v) and methanol (100 % v/v) to obtain 50 sub fractions (10 mL each). After observing TLC patterns of fractions, fractions which gave similar TLC patterns were pooled and the combined fractions were tested for anti proliferative activity. The most active combined fraction (fraction 22- 28) obtained was further separated in a silica column (152 mg) with hexane- ethyl acetate (90: 10- 0:100 v/v) to obtain 50 sub fractions (10 mL each) and tested for anti-proliferative effects by MTT assay. Active white color crystals formed in the fraction 12 were collected and purity was confirmed by normal phase TLC analysis (TLC mobile phase- Chloroform-Ethyl acetate: 7:3).

2.4 Structure Elucidation

Structure of the isolated active compound was elucidated by means of UV and IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR (COSY, HSQC, and HMBC) and ESI-MS techniques. Comparative physical and spectroscopic data is shown below (Table 1):

Hopenone-I (Observed data): Colorless solids; mp 199-201°C; EIMS *m*/*z* 424 $[M]^+$ (23), 409 (18), 381 (100), 202 (12), 188 (14), 175 (16), 161 (36), 148 (17), 133 (27), 119 (19).

Hopenone-I (Reported data): Colorless solids; mp mp 195-197°C, EIMS *m*/*z* 424 $[M]^+$ (17), 409 (14), 381 (100), 202 (9), 188 (10), 175 (11), 161 (31), 148 (12), 133 (23), 119 (16).

Table 1. Comparative NMR-chemical shift values

Observed data of hopenone-I			Reported data of hopenone-I	
C. No.	***δc***	***δH* (*J*, Hz)**	***δc***	***δH* (*J*, Hz)**
1	39.6	1.35, 1.92 m	40.0	1.93 m
2	34.1	2.35, 2.39 m	34.4	2.41, 2.48 m
3	218.2	-	218.6	-
4	48.1	-	47.6	-
5	54.8	1.34 overlap	55.1	Not reported
6	19.7	1.41, 2.25	20.0	Not reported
7	32.7	1.25, 1.50	33.0	Not reported
8	41.5	-	41.9	-
9	50.1	-	50.4	-
10	36.8	-	37.1	-
11	21.9	1.29, 1.54 m	22.2	Not reported
12	24.0	1.38, 1.49	24.3	Not reported
13	49.3	-	49.7	-
14	42.0	-	42.3	-
15	31.7	1.23, 1.26 m	32.1	Not reported
16	19.6	1.24, 2.27 m	20.0	2.26
17	139.6	-	140.0	-
18	49.7	-	50.0	-
19	41.6	1.31, 1.64 m	42.0	Not reported
20	27.4	2.10, 2.91 m	27.7	2.10, 2.18
21	136.2	-	136.5	-
22	26.4	2.49 sept (6.5)	26.6	2.62 sept (6.5)
23	26.6	1.16 s	26.9	1.07 s
24	21.0	1.19 s	21.3	1.01s
25	15.9	0.98 s	16.3	0.91 s
26	16.0	1.05 s	16.4	0.96 s
27	14.8	1.01 s	15.1	1.03 s
28	19.0	0.74 s	19.3	0.83 s
29	21.9	0.90 d (6.5)	22.1	0.90 d (*J* = 7.0 Hz)
30	21.2	0.93 d (6.5)	21.5	0.97 d (*J* = 7.0 Hz,

2.5 Cell Culture

HepG2 (hepatocellular carcinoma), MCF-7 (estrogen receptor positive breast cancer), and AN3CA (endometrial cancer) cells were harvested by trypsinization and plated in 96-well cell culture plates in Dulbecco's Modified Eagle Medium (DMEM) for 24 h at 37°C at 95% air and 5% CO_2 atmosphere with 95% humidity.

2.6 Anti Proliferative Activity Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide- MTT)

MTT assay was carried out according to the method described by Samarakoon et al. [9]. Briefly, plated cancer cells were exposed to different concentrations of compound 1 and incubated for 24 and 48 h. MTT reagent (20 µL) was then added to each well and then plates were incubated for four hours at 37°C. After the incubation, medium was removed and 100 µL of isopropanol/HCl mixture was added. Plates were shaken in a plate shaker for 30 min. Synergy™ HT Multi-Mode microplate reader (Bio- Tek, Winooski, VT) was used to measure absorbance of treated (A) and untreated (B) wells at 570 nm. Paclitaxel was used as the positive controls.

2.7 Morphological Observations under Phase Contrast Microscope

Morphological changes in compound 1 treated cancer cells were observed under phase contrast microscope (OPTIKA, XDS 3, Italy).

2.8 Data Analysis

Results are expressed as mean±SD of three independent experiments. IC_{50} values of compound 1 on different cancer cells were analysed by GraphPad Prism 5 (San Diego, California, USA) software package.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white needles; mp 199-201°C (lit. 195-199°C). The IR spectrum displayed absorptions at 2931 (CH), 1705 (C=O), and 1637 (C=C) cm^{-1}. The UV spectrum showed maximum absorptions at 244 and 208 nm. The EI-MS spectrum which showed M^+ at *m/z* 424 and base peak at *m/z* 381. The ^{1}H-NMR spectrum displayed resonances for eight methyls at δ 0.74 (3H, s, H-28), 0.90 (3H, d, *J* = 6.5 Hz, H-29), 0.93 (3H, d, *J* = 6.5 Hz, H-30), 0.98 (3H, s, H-25), 1.01 (3H, s, H-27), 1.05 (3H, s, H-26), 1.16 (3H, s, H-23), 1.19 (3H, s, H-24). The ^{13}C-NMR spectra (BB and DEPT) displayed resonances for 30 carbons including eight methyls, ten methylene, four methine and eight quaternary carbons. Resonances on ^{13}C-NMR spectrum at δ 218.2, 139.6 and 136.2 were assigned for C-3, C-17, and C-21 respectively. Four methine carbons resonated at δ 54.8, 50.1, 49.3, and 26.3 were assigned to C-5, C-9, C-13 and C-22 respectively. The structure of the compound was further confirmed using 2D-NMR spectra (COSY, HSQC, HMBC and NOESY). Position of quaternary carbons and functional groups was assigned with the help of HMBC correlations. Structure and Key HMBC correlations of compound **1** are shown in Fig. 1.

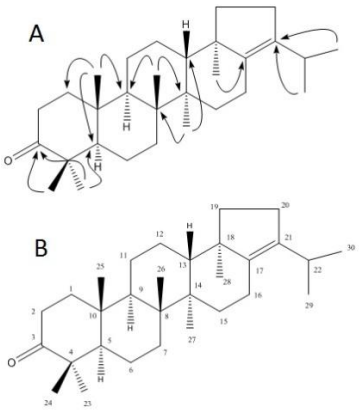

Fig. 1. [A] Key HMBC correlations in compound 1 and [B] structure of compound 1 (hopenone I)

All the spectral data of the compounds unambiguously matched with the reported compound hopenone –I [10]. This is the first report of isolation of hopenone-I from the mangrove plant *S. hydrophyllacea* despite it being isolated from other plants [11,12]. According to the results obtained from anti proliferative studies as shown in Fig. 2, hopenone-I showed some promising anti proliferative effects as assessed by MTT assay in three cancer cell lines (MCF-7, HepG2 and AN3CA) in a time and dose dependent manner { HepG2: 29.24 µM (at 24 h post incubation) and 11.60 µM (at 48 h post incubation), MCF-7: 14.98 µM (at 24 h post incubation) and 7.76 µM (at 48 h post incubation), AN3CA: 12.15 µM (at 24 h post incubation) and 4.99 µM (at 48 h post incubation)}.

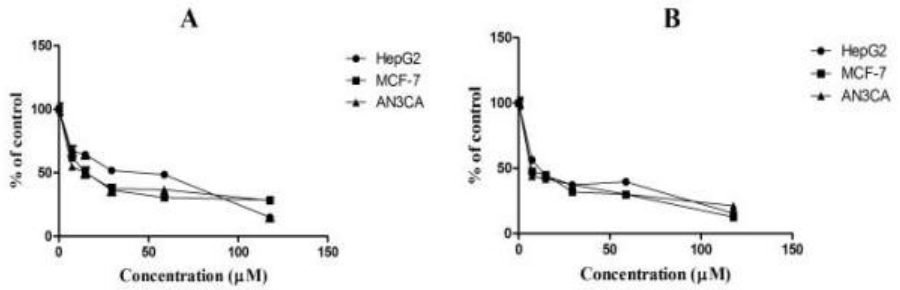

Fig. 2. Dose and time dependent cytotoxic effects of hopenone-I isolated from the leaves of mangrove plant *S. hydrophyllacea*. [A] 24 h incubation [B] 48 h incubation

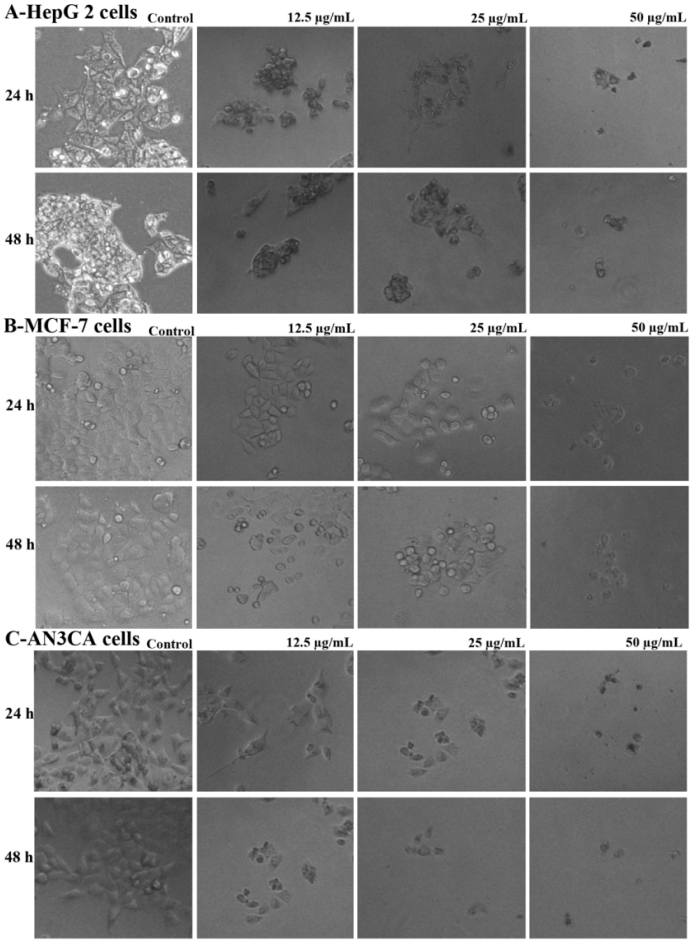

Fig. 3. Morphological changes observed under phase contrast microscope in hopenone -I treated [A] HepG2, [B] MCF-7, and [C] AN3CA cells

Changes in cell shape, structure and decrease in cell number were also observed under phase contract microscope in hopenone-I treated cancer cells when compared to untreated controls as shown in Fig. 3. Collectively, findings of this study identified for the first time hopenone-I as one of the active anti proliferative compounds present in *S. hydrophyllacea* and its anti-proliferative properties in cancer cells. However more molecular mechanism based studies are needed to exploit anti-cancer properties of hopenone-I in cancer cells.

4. CONCLUSION

According to the results of the present study, we conclude that hopenone –I isolated for the first time from the leaves of *S. hydrophyllacea* may be useful as an anti proliferative agent targeting cancer.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod. 1977;60:52-60.
2. American Cancer Society. Global cancer facts and figures, 3rd edition, Atlanta: American cancer society; 2015.
3. Howard JH, Bland KI. Current management and treatment strategies for breast cancer. Curr. Opin. Obstet. Gynecol. 2012;24(1):44-8.
4. Thompson A, Brennan K, Cox A, Gee J, Harcourt D, Harris A, Harvie M, Holen I, Howell A, Nicholson R, Steel M, Streuli C. Evaluation of the current knowledge limitations in breast cancer research: A gap analysis. Breast Cancer Res. 2008; 10(2):R26.
5. Johnson IT. Phytochemicals and cancer. Proc Nutr Soc. 2007;66(2):207-15.
6. Feng CL, Gong MF, Zeng YB, Dai HF, Mei WL. Scyphiphin C, a new iridoid from *Scyphiphora hydrophyllacea*. Molecules. 2010;15(4):2473-7.
7. Zeng YB, Mei WL, Zhao YX, Dai HF. Two new noriridoids from *Scyphiphora hydrophyllacea.* Naturforsch. 2008;63b: 108−110.
8. Zeng YB, Mei WL, ZhaoYX, Dai HF. Cytotoxic components from mangrove plant *Scyphiphora hydrophyllacea*. J. Trop. Subtrop. Bot. 2007;15:249−252.
9. Samarakoon SR, Thabrew I, Galhena PB, De Silva D, Tennekoon KH. A comparison of the cytotoxic potential of standardized aqueous and ethanolic extracts of a polyherbal mixture comprised of Nigella sativa (seeds), Hemidesmusindicus (roots) and Smilax glabra(rhizome). Pharmacognosy Res. 2010;2:335–342.
10. Shen CC, Syu WJ, Li SY, Lin CH, Lee GH, Sun CM. Antimicrobial activities of naphthazarins from *Arnebia euchroma.* J. Nat. Prod. 2002;65:1857-1862.
11. Hui WH, Li MM. Six new triterpenoids and other triterpenoids and steroids from three Quercus species of Hong Kong. J. Chem. Soc., Perkin Trans.1. 1977;8:897-904
12. Hui WH, Li MM. Structures of eight new triterpenoids and isolation of other triterpenoids and epi-ikshusterol from the stems of Lithocarpus cornea. J. Chem. Soc., PerkinTrans.1. 1976;23-20.

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