



## **Fish Singgang Extracts as a Potential Anti-Proliferative against Colon Cancer Cell Lines (HT-29, HCT-116, CT-26)**

**Anis Nafisah Jamain<sup>1</sup>, Nur Atikah Anwar<sup>1</sup>, Norhaslinda Ridzwan<sup>1</sup>,  
Mimie Noratiqah Jumli<sup>1</sup>, Norhayati Abd Hadi<sup>1</sup>, Roslan Arshad<sup>2</sup>,  
Mohd Adzim Khalili Rohin<sup>1\*</sup>, Che Abdullah Abu Bakar<sup>2</sup>  
and Ahmad Zubaidi A. Latif<sup>3</sup>**

<sup>1</sup>*School of Nutrition & Dietetics, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300, Kuala Nerus, Terengganu Darul Iman, Malaysia.*

<sup>2</sup>*Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu Darul Iman, Malaysia.*

<sup>3</sup>*Faculty of Medicine, Universiti Sultan Zainal Abidin, Medical Campus, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu Darul Iman, Malaysia.*

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors AZAL, CAAB and MAKR designed the study concept and design. Authors RA and NAH design the methodology. Authors ANJ, NAA, NR, RA and MNJ carried out the experimental, data acquisition and data interpretation. Authors ANJ and NR drafting the manuscript. Authors AZAL, CAAB and MAKR revised critically on the manuscript written. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JPRI/2021/v33i29B31597

Editor(s):

(1) Prof. Paula Mendonça Leite, Universidade Federal de Minas Gerais, Brazil.

Reviewers:

(1) S. Hemalatha, B. S. Abdur Rahman Crescent Institute of Science and Technology, India.

(2) Stefania Lamponi, University of Siena, Italy.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67956>

**Original Research Article**

**Received 05 March 2021**

**Accepted 10 May 2021**

**Published 25 May 2021**

### **ABSTRACT**

**Aim:** To investigate anti-proliferative effect of three types of Terengganu singgang extracts on colon cancer cell lines (HT-29, HCT-116, CT-26).

**Study Design:** Experimental study.

**Place and Duration of Study:** Central Laboratory, Tissue Culture Laboratory, Universiti Sultan Zainal Abidin, Terengganu between April 2019 and July 2019.

**Methodology:** Samples comprised three types of singgang dish, which were prepared, cooked, and then extracted with distilled water and ethanol (EtOH) in different strengths, 50%, 70%, and 100%. These singgang samples were chub mackerel (ST), Indian mackerel (SK), and a control sample with no fish (SC). Extracts were analyzed for their anti-proliferative effect by MTT-based assay. Then, the morphological of cell apoptotic changes was observed using light inverted microscope.

**Results:** Experimental assays showed that the SC sample extracted in 100% EtOH produced the highest yield (3.7%). The extract of ST in aqueous (0.27 (0.11)) yielded the most cytotoxic value, followed by extract SK in 100% EtOH (0.28 (0.10)) and extract SC in 50% EtOH (0.20 (0.08)). Then, the anti-proliferative effect was confirmed with morphological changes of cell which were characterized by cell shrinkage, membrane blebbing, and fragmentation of apoptotic bodies after 24, 48 and 72 hours of treatment.

**Conclusion:** In conclusion, the ST extract showed the best anti-proliferative effect.

*Keywords: Anti-proliferative; singgang dish; colorectal cancer; MTT assay.*

## 1. INTRODUCTION

In Malaysia, colorectal or colon cancer is the second most common cancers between 2012 – 2016, most common cancer among males (16.9%) and second most common in women (10.7%) [1]. According to the National Cancer Registry Report 2012 – 2016, Malaysia reported a significant increase in the incidence of colorectal cancer by 5%; with 13.50 per 100,000 [2]. Most patients with colorectal cancer have been diagnosed at a late stage in Malaysia, with the 5-year relative survival lower than that in Asian countries [3]. Over the time, the economic burden of colorectal cancer and the low efficacy of treatment is likely to increase due to current trend and aging population [4]. Therefore, alternative sources are of particular interest and have been studied extensively nowadays as anticancer agents because they are usually linked with low toxicity profiles.

In Asian, spices and herbs such as turmeric, galangal, garlic, sour plum and chillies are well known flavour enhancers for food. In general, turmeric is known to have antioxidant, antibacterial, anti-inflammatory, antiviral, antifungal, and anticancer activity [5]. Meanwhile, galangal has the antioxidant, anti-cancer, anti-inflammatory, anti-fungal and anti-diabetic potential [6]. Also, garlic has antioxidant, anti-carcinogenic, hypolipidemic effects while improving our body's immune function [7]. Then, sour plum also could provide health benefits on anti-proliferative, antioxidant, anti-inflammatory, anti hyperlipidemic [8] and anti-obesity effect that acts as an inhibitor of lipogenesis [9-10]. On the other hand, chillies also had been known to have anti-cancer, antioxidant, anti-hypertension and hypolipidemic effects [11].

In Terengganu, singgang is a signature and traditional fish dish, commonly cooked by boiling the mackerel (chub mackerel or Indian mackerel) with selected spices such as turmeric, galangal, garlic, chillies and sour plum. Nevertheless, studies had reported that consumption of fish can reduce the risk of getting colorectal cancer as fish had n-3 long chain fatty acids and rich in omega-3 and omega-6 contents [12-13], that could induce cell death to tumor cell via apoptotic pathway and interfering the components of cell cycle that can alter the growth of tumor cells [13]. As selected spices, herb and fish had been aforementioned on their potential anti-proliferative and anti-cancer effect, it is believed that the singgang dish would provide nutritious meals with high potential anti-proliferative effect. Therefore, this study is aimed to investigate the anti-proliferative effect of fish singgang dish against colon cancer cell lines.

## 2. MATERIALS AND METHODS

### 2.1 Sample Preparation from Singgang Dish

In this study, dish of singgang were prepared at the Therapeutic Diet and Laboratory of Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Terengganu, representing three types of samples namely, the chub mackerel singgang (ST), Indian mackerel singgang (SK), and the control singgang (SC). The SC sample was prepared without fish, i.e., comprising herbs and spices only. The herb and spices of each sample comprised of 15 g ground turmeric, 15 g ground galangal, 25 g fresh chillies, 6 g garlic, and 10 g sour plum, and simmered in 600 mL distilled water for 5 min. Then, 5 g salt and 3 g sugar were added and followed by 500 g chub

mackerel and 500 g Indian mackerel to ST and SK, respectively. Upon seasoning for 2 min, each singgang dish was boiled for 20 min, after which 400 g of the edible portion (fish and gravy) of singgang dish was blended using a kitchen blender (HR2027/75, Koninklijke Phillips, N. V.) for 2 min to generate a homogenous mixture for each sample (ST, SK, and SC). The homogenous mixture was stored at  $-20^{\circ}\text{C}$  until the nutrient extraction.

The extraction was largely based on the method of Mohd Adzim Khalili et al. [14], in which 30 g of each blended singgang dish sample (ST, SK, SC) was soaked in four different solvents, i.e., 100% ethanol (EtOH), 70% EtOH, 50% EtOH, and distilled water at room temperature for 24 h at an extracting ratio of sample to solvent 1: 10 (w/v). Altogether, 12 samples (three types of singgang x four solvents) were prepared. The supernatants of each sample were then filtered with nylon filter papers (pore size  $0.45\ \mu\text{m}$ ) and evaporated using a rotary evaporator (BUCHI, R-215, Labortechnik AG) connected to a vacuum pump for 60 min at a reduced pressure (2300 - 5830 Pa) at  $40^{\circ}\text{C}$  to yield the crude extract. The crude extract of each sample was dried in a drying oven at  $40^{\circ}\text{C}$  for 60 min and then frozen at  $-20^{\circ}\text{C}$  before chemical analysis. The extraction yield (Y) was calculated [15].

## 2.2 In-vitro Study

### 2.2.1 Preparation of singgang extracts

The methods used previously by Khalili et al. [16] were adopted for this study with slight modification. A total of 10 mg of different singgang extracts were dissolved in 1 mL of DMSO to prepare a stock solution of 10 mg/mL. The extract solutions were kept at  $4^{\circ}\text{C}$  before use. The stock solution for each extract was further diluted in completed RPMI-1640 media added with foetal bovine serum (10%) and penicillin-streptomycin (1%) to obtain a working solution of 1 mg/mL.

### 2.2.2 Cell maintenance and harvesting

Colon cancer cell lines of human colorectal adenocarcinoma, HT-29 (ATCC® HTB-38™), human colorectal carcinoma, HCT-116 (ATCC® CCL-247™), and mouse colorectal carcinoma, CT-26 (ATCC® CRL-2638™) were used in this study. The cells were obtained at passage 3 (P3) from the Faculty of Bioresources and Food Industry, UniSZA. The cell lines were grown and

maintained in completed RPMI-1640 added with foetal bovine serum of 10% and penicillin-streptomycin of 1% at  $37^{\circ}\text{C}$  in an incubator humidified with 5%  $\text{CO}_2$  and relative humidity of 95%. The cell media was replaced twice weekly to replenish the nutrients required for cell growth.

### 2.2.3 Determination of inhibitory concentration at 50% ( $\text{IC}_{50}$ )

The inhibitory concentration ( $\text{IC}_{50}$ ) of singgang extracts was evaluated using a colorimetric micro-titration method known as the MTT assay or tetrazolium salt reduction assay [17]. The cells were harvested from the media, counted using a haemocytometer, and further diluted in a completed RPMI medium (added with 10% foetal bovine serum and 1% penicillin-streptomycin). A total of 100  $\mu\text{L}$  of cell suspension was seeded in triplicates using 96-well culture plates (SPL Life Sciences, Korea) at an optimized density of  $1 \times 10^5$  cells/ $\text{cm}^2$  for each cell line. After 24 hours, triplicate serial dilutions of singgang extracts (1 - 0.0313 mg/mL) [18] and doxorubicin (drug) (1.00 - 0.016  $\mu\text{g}/\text{mL}$ ) were added into each well. Each 96-well plate was equipped with blank cells (blank) and untreated cells (positive control). After a 72-hour incubation period, 20  $\mu\text{L}$  (5  $\mu\text{g}/\text{mL}$ ) of MTT assay was added into each well in 96-well plate and kept for an additional 4 hours. The medium was discarded and 100  $\mu\text{L}$  of DMSO reagent was further into each well. Next, the absorbance at 570 nm with reference to 630 nm was measured using a microplate reader (TECAN, INFINITEM200, Switzerland). Appropriate controls for the determination of cell viability were also measured. The relative cell viability of the treated cells was described as %cell viability and calculated based on the following formula:  $(A_{570}$  of treated cells/  $A_{570}$  of control cells) x 100% and calculated to depend on the non-linear regression of the response curves within the same region.

### 2.2.4 Cell morphology observation

The effect of singgang extracts on the cellular morphological changes were determined using the method by Merlin et al. [19]. The HCT-116, HT-29 and CT-26 cells were grown and treated with the  $\text{IC}_{50}$  value of each singgang extract as an effective dosage concentration. The morphological changes were observed at  $37^{\circ}\text{C}$  for 24, 48, and 72 hours using a light inverted microscope (Nikon, Japan) at magnification 20x [18].

## 2.3 Statistical Analysis

Both descriptive and inferential statistical analyses were used to analyze the data. The software Statistical Package for Social Sciences (SPSS, version 20.0, IBM, Armonk, USA) was used to perform two-tailed tests at the significance level of 0.05.

## 3. RESULTS AND DISCUSSION

### 3.1 Extraction Yield of Singgang Extracts

Table 1 shows the extraction yield (%) of SK, ST, and SC samples; the difference yield between original sample and evaporated dried extract in four different solvents. For SK, 70% EtOH gave the highest extraction yield (3.54%), and followed by 50% EtOH, 100% EtOH, and distilled water with 2.84%, 2.56%, and 2.26% yield, respectively ( $p < 0.05$ ). For ST, 70% EtOH gave the highest extraction yield (3.45%), and followed by 50% EtOH, distilled water, and 100% EtOH with 3.32%, 2.63%, and 2.49% yield, respectively ( $p < 0.05$ ). Meanwhile, the highest extraction yield for SC was shown by 100% EtOH and followed by 70% EtOH, 50% EtOH, and distilled water with 3.74%, 3.51%, 3.06%, and 2.10% yield, respectively ( $p < 0.05$ ). Overall, the SC sample extracted in 100% EtOH gave the highest yield among all samples (3.74%).

Also, extractions in distilled water gave a significantly lower yield than that of 100%, 70%, and 50% EtOH for each sample. In contrast, extraction in 70% EtOH gave a significantly higher ( $p < 0.05$ ) yield for ST and SK samples

than 100% EtOH. While, 50% EtOH similarly gives higher yield in SK, ST and SC samples compared to distilled water. In general, the yield of extractions in organic solvents such as acetone, methanol, and ethanol would be improved at higher water content because the extracted compound might be soluble in both water and organic solvent [20]. However, contradicted results of the study indicated several parameters such as sample particle size, chemical composition of the phytochemicals and others might affected overall result [20-21].

### 3.2 Anti-proliferative Effect of Fish Singgang Extracts

Table 2 shows the anti-proliferative activities of singgang extracts on HT-29, HCT-116 and CT-26 cells using MTT assay. For ST sample, distilled water extract gives high anti-proliferative effect with  $IC_{50}$  0.27 (0.11) mg/mL to HCT-116 cells, while 70% EtOH extract gives low anti-proliferative effect with  $IC_{50}$  0.96 (0.13) mg/mL to CT-26 cells. While, SK of 100% EtOH extract gives high anti-proliferative effect with  $IC_{50}$  0.28 (0.10) mg/mL to HCT-116 cells, while 50% EtOH extract gives low anti-proliferative effect with  $IC_{50}$  0.92 (0.17) mg/mL to CT-26 cells. Follows, SC sample observed the 50% EtOH extract gives the high anti-proliferative effect with  $IC_{50}$  0.20 (0.08) mg/mL to HCT-116 cells, while low anti-proliferative effect was observed with  $IC_{50}$  0.90 (0.14) mg/mL by 100% EtOH extract to CT-26 cells. Thoroughly, ST sample of distilled water, SK sample of 100% EtOH, and SC sample of 50% EtOH provides high anti-proliferative effect.

**Table 1. Extraction yield of singgang extracts using 100% EtOH, 70% EtOH, 50% EtOH and distilled water**

Samples	Solvents	Extraction yield (%)	F statistics (df)	p value
ST	100% EtOH	2.63 (0.01)	11.81 (3,8)	<0.001*
	70% EtOH	3.45 (0.01)		
	50% EtOH	3.32 (0.01)		
	distilled water	2.49 (0.01)		
SK	100% EtOH	2.56 (0.01)	99.59 (3,8)	<0.001*
	70% EtOH	3.54 (0.01)		
	50% EtOH	2.84 (0.01)		
	distilled water	2.26 (0.01)		
SC	100% EtOH	3.74 (0.01)	46.97 (3,8)	<0.001*
	70% EtOH	3.51 (0.01)		
	50% EtOH	3.06 (0.01)		
	distilled water	2.10 (0.01)		

Data are expressed as mean (standard deviation). Values shown are means of 3 independent experiments  
\*Post hoc analysis: the extraction yield is statistically different from each other

In the present study, each of samples ST, SK and SC showed the higher anti-proliferative effect with the IC<sub>50</sub> values of 0.27 (0.11), 0.03 (0.10), and 0.20 (0.08) mg/mL, respectively. Based on the United States National Cancer Institute Plant Screening Program, IC<sub>50</sub> values of 20 µg/ml or less in plant extract can be recognized as having active cytotoxic effect during the incubation period between 48 and 72 hours [22]. Nevertheless, the results from the present study only supported on anti-proliferative instead of cytotoxicity effects of singgang dish towards colon cancer cell lines (HCT-116, HT-29, CT-26). The results might be due to the synergistic effects of the spices and herbs used in the cooking of singgang dish. However, the benefits of anti-proliferative effects of each spice and herbs used in this study such as garlic, galangal, sour plum and chillies had thoroughly been studied previously [5 –8,11].

On the addition, the study observed HCT-116 cells as the most sensitive cell, while CT-26 cells as the least sensitive cell for treatment samples ST, SK and SC. Though, there is no significant difference in IC<sub>50</sub> values of different cell lines among samples ST, SK and SC ( $p > 0.05$ ) (data not shown). The result had been supported by Koosha et al. [23], which discovered that HCT-116 were more sensitive to diosmetin (type of bio-flavonoid) than HT-29 and CCD-841 cells (normal colon tissue) by exhibiting higher cytotoxic effect. However, Zheng et al. [24] found that HCT-116 cells were less sensitive than HT-29 cells after being treated with protopanaxadiolgin sinusoids (compounds in ginseng). Nevertheless, another study discovered that short term effect and high sensitivity of HCT-116 cells towards treatment is due to its higher and faster growth rate compared to other cell lines (HT-29, CCD-18Co) [25]. The findings also found that short term of drug treatment including oxaliplatin or docetaxel in HCT-116 cells had more detectable changes in cell viability than HT-29 cells.

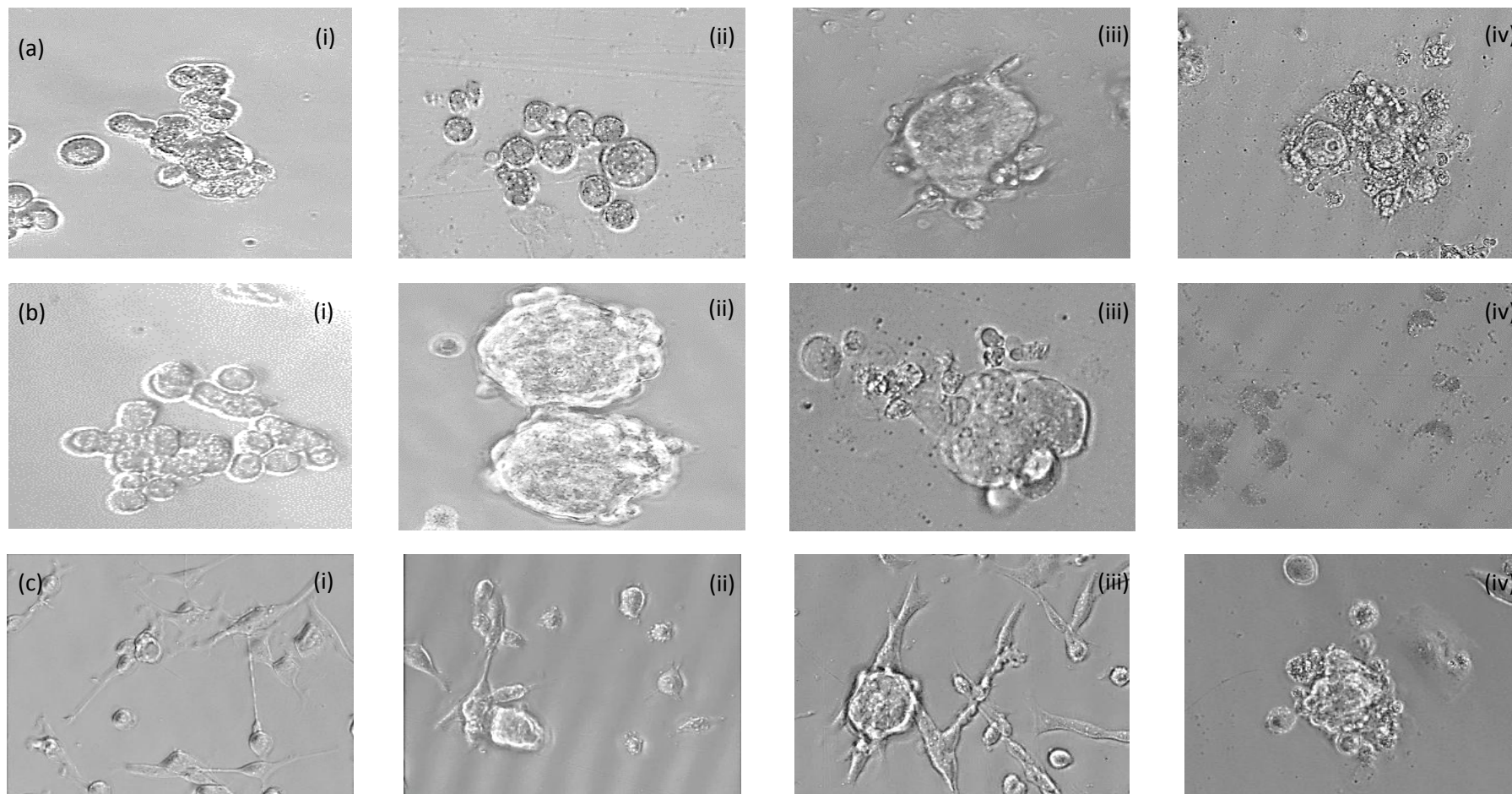
Previously, the cytotoxic effect of turmeric had been mediated through apoptosis inducing tumor suppressor protein by decreasing the pro-survival COX-2 and Cyclin D1 which could cause more tumor formation [26]. While, garlic with its bioactive compounds, namely; selenomethionine and se-methyl-L-selenocysteine had provided the anti-proliferative effect by activating extrinsic apoptotic pathway and down-regulating cell cycle progression [27]. Besides, capsaicin from chillies

also exhibited anti-proliferative effect of cell cycle arrest at G<sub>0</sub>/G<sub>1</sub> phase and increase the synthesis of pro-apoptotic protein to induce apoptotic cell death [28]. Comprehensively, the anti-proliferative effect of one dish contains herbs, spices and protein sources had not been studied yet as there is a tremendous report on individually benefits towards cancer cell lines. Still, Vemuri et al. [29] had studied the combination of natural spices comprised of turmeric, ginger and garlic towards MCF-7 and observed that the combination extract of the spices gives higher anti-proliferative effect with tamoxifen drug added compared to each spices or tamoxifen drug effect in MCF-7 cells. Similarly, the combination of spices, herbs and protein sources in singgang dish had observed its anti-proliferative effect towards colon cancer cell lines in the present study.

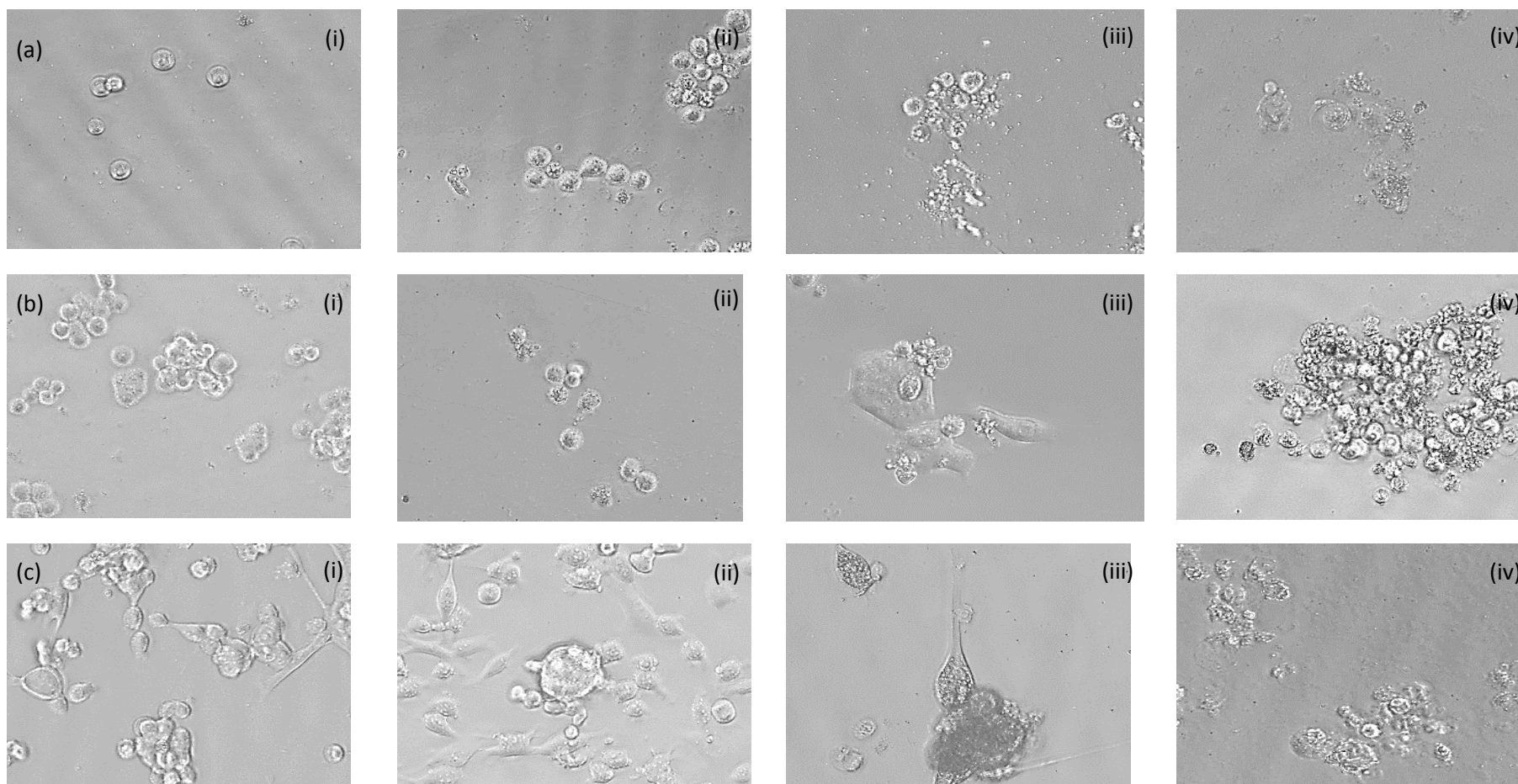
### 3.3 Detection of Morphological Changes in Colon Cancer Cells by Fish Singgang Extracts

The treated cells of HT-29, HCT-116 and CT-26 with IC<sub>50</sub> values samples of ST, SK and SC (100% EtOH, 70% EtOH, 50% EtOH, distilled water) were incubated for 72 h and the morphological changes effect were compared with the untreated cells, as been depicted in Fig. 1, 2 and 3. Under control conditions at 0 h, HT-29, HCT-116 and CT-26 cells appeared healthy with growth up to 90% confluences of the cell. The HT-29 and HCT-116 cells were round and intact when compared to CT-26 cells, which displayed a polygonal and branching shape, thus reflecting the normal growth patterns for each of the colorectal cancer cell lines. Based on Latifah et al. [30], apoptotic hallmarks were noticed such as cell shrinkage and round off, membrane blebbing, nuclear condensation, nuclear fragmentation, detachment of cells from the surface, and the presence of apoptotic bodies and cell debris.

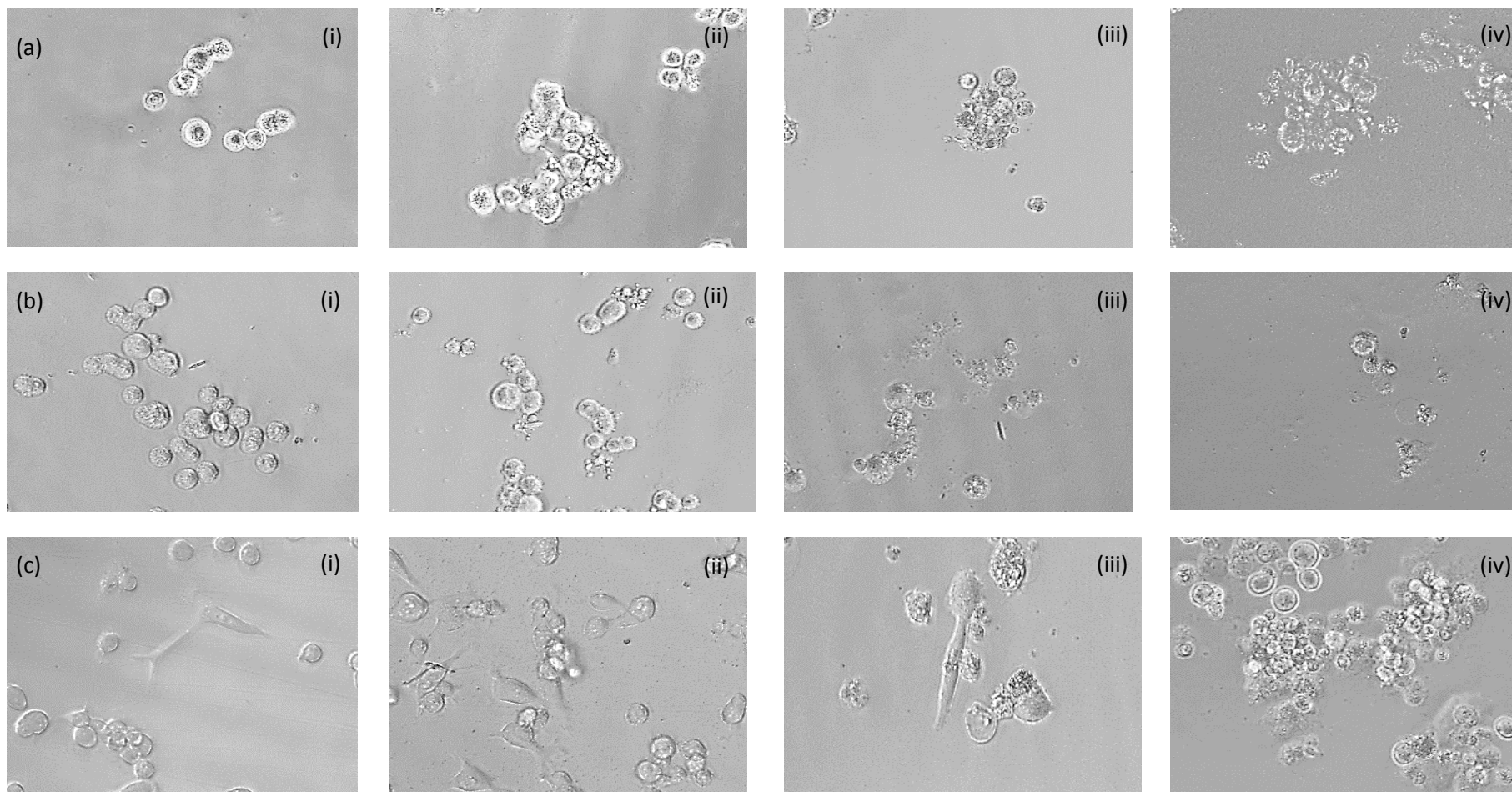
At 24 h treated, observed HT-29, HCT-116 and CT-26 cells became condensed, shrinkage and some of the cells undergone partial detached from the surface and there was a decline in the cell volume due to round off of the cells Fig. 1, 2, 3 – (a, ii) (b, ii) (c, ii). Then at 48 h, there were presence of membrane blebbing (cytoplasmic protrusions) with some small cytoplasmic package (also called apoptotic bodies) and some of the organelles became too compact and appeared very condensed Fig. 1, 2, 3 – (a, iii) (b, iii) (c, iii). At 72 hours, the treated cells became



**Fig. 1.** Inhibition of (a) HT-29, (b) HCT-116 and (c) CT-26 by ST extracts of 100% EtOH, 70% EtOH, 50% EtOH and distilled water for 3 days. Cell morphology of HT-29, HCT-116 and CT-26 were examined after being treated with  $IC_{50}$  at (i) 0 hour, (ii) 24 hours (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan)



**Fig. 2.** Inhibition of (a) HT-29, (b) HCT-116 and (c) CT-26 by SK extracts of 100% EtOH, 70% EtOH, 50% EtOH and distilled water for 3 days. Cell morphology of HT-29, HCT-116 and CT-26 were examined after being treated with  $IC_{50}$  at (i) 0 hour, (ii) 24 hours (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan)



**Fig. 3. Inhibition of (a) HT-29, (b) HCT-116 and (c) CT-26 by SC extracts of 100% EtOH, 70% EtOH, 50% EtOH and distilled water for 3 days. Cell morphology of HT-29, HCT-116 and CT-26 were examined after being treated with  $IC_{50}$  at (i) 0 hour, (ii) 24 hours (iii) 48 hours and (v) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan)**



**Table 2. Anti-proliferative effect of fish Singgang extracts on HT-29, HCT-116, CT-26**

Samples	Solvents	IC <sub>50</sub> (mg/ml)			F-statistics (df)	p-value
		HCT-116	HT-29	CT-26		
ST	100% EtOH	0.31 (0.04)	0.42 (0.05)	0.95 (0.02)	5.937 (2,9)	<0.001*
	70% EtOH	0.47 (0.17)	0.41 (0.12)	0.96 (0.13)		
	50% EtOH	0.37 (0.15)	0.39 (0.16)	0.93 (0.13)		
	distilled water	0.27 (0.11)	0.32 (0.15)	0.73 (0.10)		
SK	100% EtOH	0.28 (0.10)	0.75 (0.12)	0.81 (0.17)	2.372 (2,9)	0.149
	70% EtOH	0.56 (0.13)	0.51 (0.14)	0.55 (0.21)		
	50% EtOH	0.50 (0.12)	0.43 (0.13)	0.92 (0.17)		
	distilled water	0.45 (0.10)	0.47 (0.13)	0.49 (0.12)		
SC	100% EtOH	0.30 (0.13)	0.81 (0.14)	0.90 (0.14)	0.786 (2,9)	0.485
	70% EtOH	0.31 (0.11)	0.44 (0.15)	0.65 (0.13)		
	50% EtOH	0.20 (0.08)	0.29 (0.16)	0.48 (0.18)		
	distilled water	0.87 (0.11)	0.40 (0.17)	0.50 (0.15)		

Data are expressed as mean (standard deviation), \*Post hoc analysis: the IC<sub>50</sub> value is statistically different from each cell

lobulated due to loss of normal cell shape, presence of cell fragmentation and cell debris, some of the cell lysed, presence of apoptotic bodies, and some of the cell were in crescent shaped indicated as final stage of apoptosis Fig. 1, 2, 3 – (a, iv) (b, iv) (c, iv).

#### 4. CONCLUSION

Overall, the SC sample extracted in 100% EtOH gave the highest extraction yield with 3.74% among other sample extracted. Meanwhile, ST sample extracted in distilled water gives high anti-proliferative effect with IC<sub>50</sub> 0.27 (0.11) mg/mL, followed by SK sample extracted in 100% EtOH (0.28 (0.10)) mg/mL and SC sample extracted in 50% EtOH (0.20 (0.08)) mg/mL. On the other hand, ST sample extracted in 70% EtOH gives low anti-proliferative effect with 0.96 (0.13) mg/mL, followed by SK sample extracted in 50% EtOH (0.92 (0.17) mg/mL) and SC sample extracted in 100% EtOH (0.90 (0.14) mg/mL). The anti-proliferative effect were confirmed with apoptotic hallmarks such as cells shrinkage, membrane blebbing, nuclear condensation, detachment of cells and presence of apoptotic bodies and cells debris after treated on 24, 48 and 72 hours. Nevertheless, further investigation into the analysis of phytochemicals contributing to the anti-proliferation effect in the singgang dish would be essential.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### ACKNOWLEDGEMENTS

This study was supported by grant number UniSZA/RMIC/2018/04 from the Centre for Research, Excellence and Incubation Management (CREIM) at Universiti Sultan Zainal Abidin. A special thankful for the help provided by Dean of Faculty Medicine; Prof Dr. Nordin Simbak for guiding us throughout the study. Special thanks also to all collaborative researchers whom participated in this study and had given full cooperation in providing the necessary input and equipment. The acknowledge also goes to all staffs from Teaching Laboratory 1, Faculty of Medicine and Faculty of Health Sciences, UniSZA.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. TheStar. 2020. Colon cancer is second most common in Malaysia; 2020. Available: <https://www.thestar.com.my/news/nation/2020/03/10/colon-cancer-is-second-most-common-in-msia> [16 August 2020]
2. Code Blue. 2020. Cancer cases rise in Malaysia, Chinese most prone; 2020. Available: <https://codeblue.galencentre.org/2020/01/03/cancer-cases-rise-in-malaysia-chinese-most-prone/> [16 August 2020]
3. Veettil SK, Lim KG, Chaiyakunapruk N, Ching SM, Abu Hassan MR. Colorectal

- cancer in Malaysia; its burden and implications for a multi-ethnic country. *Asian Journal of Surgery*. 2017;40(6):481-489.
4. Magaji B, Moy FM, Roslani A, Law CW. Survival rates and predictors among colorectal cancer patients in a Malaysian tertiary hospital. *BMC Cancer*. 2017;17(1): 1-4.
  5. Rahmani AH, Alsahli MA, Aly SM, Khan MA, Aldebasi YH. Role of curcumin in disease prevention and treatment. *Advance Biomedical Research*. 2018;7: 38.
  6. Chouni A, Paul S. A review on phytochemical and pharmacological potential of *Alpinia galanga*. *Pharmacognosy Journal*. 2018;10(1):9-15.
  7. Adaki S, Adaki R, Shah K, Karagir A. Garlic: review of literature. *Indian Journal Cancer*. 2014;51:577-581.
  8. Hamidon H, Susanti D, Taher M, Zakaria ZA. *Garcinia atroviridis* – A review on phytochemicals and pharmacological properties. *Marmara Pharmaceutical Journal*. 2017;21:38-47.
  9. Jena BS, Jayaprakasha GK, Singh RP, SakariahKK. Chemistry and biochemistry of hydroxycitric acid from *Garcinia*. *Journal of Agricultural and Food Chemistry*. 2002; 50(1):10–22.
  10. Mackeen MM, Ali AM, Lajis NH, Kawazu K, Kikuzaki H, Nakatani N. Antifungal *Garcinia* acid esters from the fruits of *Garcinia atroviridis*. *Zeitschrift f'ur Naturforschung*. 2002;57(3-4):291–295.
  11. Saleh BK, Omer A, Teweldemedhin B. Medicinal uses and health benefits of chili pepper (*Capsicum* spp.): a review. *MOJ Food Processing and Technology*. 2018;6 (4):325–328.
  12. Hall MN, Chavarro JE, Lee IM, Willet WC, Ma J. 22-year Prospective study of fish, n-3 fatty acid intake, and colorectal cancer risk in men. *Cancer Epidemiology Biomarkers Prevention*. 2008;17(5):1136–1143.
  13. Huerta-Yopez S, Tirado-Rodriguez AB, Hankinson O. Role of diets rich in omega-3 and omega-6 in the development of cancer. *Boletin Medico del Hospital Infantil de Mexico*. 2016;73(6):446-456.
  14. Mohd Adzim Khalili R, Norhaslinda R, Mimie NJ, Norhayati AH, Syed Ahmad Tajudin TJ, Ahmad Zubaidi AL. Cytotoxicity study and morphological changes of different extraction for Bismillah leaf (*Vernonia amygdalina*) in human glioblastoma multiforme cell line (U-87). *Biomedical Research*. 2017;28 (2):1-7.
  15. Dey S, Rathod VK. Ultrasound assisted extraction of b-carotene from *Spirulina platensis*. *Ultrasonics Sonochemistry*. 2013; 20:271–276.
  16. Khalili RMA, Noratqah JM, Norhaslinda R, Norhayati AH, Atif AB, Roslan A. Ahmad Zubaidi AA. Cytotoxicity effect and morphological study of different Duku (*Lansium domesticum* Corr.) extract toward human colorectal adenocarcinoma cells line (HT-29). *Pharmacognosy Journal*. 2017;9(6):757-761.
  17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 1983; 65(1-2):55–63.
  18. Rohin MAK, Jumli MN, Ridzwan N, Baig AA, Latif AZA, Hadi NA. Effects of *Gynura procumbens* extracts on anti-proliferative activity and its associated morphological changes of human *glioblastoma multiforme* cell line (U-87). *Pharmacognosy Journal*. 2018;10(3):492-496.
  19. Merlin NJ, Parthasarathy V, Santhoshkumar TR. Induction of apoptosis in human breast cancer cell line MCF-7 by phytochemicals from *Gmelina asiatica*. *African Journal of Biotechnology*. 2010;9 (28):4451-4456.
  20. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*. 2014;22(3):296-302.
  21. Ridzwan N, Jumli MN, Baig AA, Rohin MAK. Quantitative and optimization of anthocyanin extracted from pomegranate (*Punica granatum*) extract by high performance liquid chromatography. *Pharmacognosy Journal*. 2018;10(1):73-82.
  22. Lee CC, Houghton P. Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. *Journal of Ethnopharmacology*. 2005;100(3):237–243.
  23. Koosha S, Mohamed Z, Sinniah A, Alshawsh MA. Investigation into the molecular mechanisms underlying the anti-proliferative and anti-tumorigenesis activities diosmetin against HCT-116

- human colorectal cancer. Scientific Reports. 2019;9(1):1-17.
24. Zheng Y, Nan H, Hao M, Song C, Zhou Y, Gao Y. Antiproliferative effects of protopanaxadiol ginsenosides on human colorectal cancer cells. Biomedical Reports. 2013;1(4):555–558.
  25. El Khy F, Corcos L, Durand S, Simon B, Le Jossic-Corcos C. Acquisition of anticancer drug resistance is partially associated with cancer stemness in human colon cancer cells. International Journal of Oncology. 2016;49(6):2558–2568.
  26. Salem M, Rohani S, Gillies ER. Curcumin, a promising anti-cancer therapeutic: a review of its chemical properties, bioactivity and approaches to cancer cell delivery. RSC Advances. 2014;4(21): 10815.
  27. Zheng J, Zhou Y, Li Y, Xu DP, Li S, Li HB. Spices for prevention and treatment of cancers. Nutrients. 2016;8(8):495.
  28. Ahmed K, Zaidi S, Cui Z, Zhou D, Saeed S, Inadera H. Potential proapoptotic phytochemical agents for the treatment and prevention of colorectal cancer (Review). Oncology Letters. 2019;18(1): 487-498.
  29. Vemuri SK, Banala RR, Subbaiah GPV, Srivastava SK, Reddy AVG, Malarvili T. Anti-cancer potential of a mix of natural extracts of turmeric, ginger and garlic: A cell-based study. Egyptian Journal of Basic and Applied Sciences. 2017;4(4): 332–344.
  30. Latifah SY, Faujan HA, Sze LP, Raha AR, Hisyam AH, Li OC. Curcumin from Turmeric (*Curcuma longa*) induced apoptosis in human mammary carcinoma cells (MDA-MB-231). Malaysian Journal of Medicine and Health Sciences. 2006;2 (2):71-79.

---

© 2021 Jamain et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle4.com/review-history/67956>