

Chemical composition, antiproliferative and antioxidant attributes of ethanolic extract of resinous sediment from *Etlingera elatior* (Jack.) inflorescence

Majed Ahmed Al-Mansoub^{1,*}, Muhammad Asif^{1,2}, Vageesh Revadigar¹,
Mohamed A. Hammad³, Nelson Jeng-Yeou Chear⁴,
Mohammad Razak Hamdan⁴, Amin Malik Shah Abdul Majid¹,
Mohd Zaini Asmawi¹, Vikneswaran Murugaiyah^{1,*}

¹Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Gelugor, Penang, Malaysia, ²Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Punjabe, Pakistan, ³Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Gelugor, Penang, Malaysia, ⁴Centre for Drug Research, Universiti Sains Malaysia, 11800, Gelugor, Penang, Malaysia

The ethanolic extract of resinous sediment (EERS) of *Etlingera elatior* young inflorescence was examined for its anticancer effect and potential antioxidant activity. The anticancer effect of the EERS was evaluated on four human cancer cell lines, HCT 116, HT-29, HeLa, and MCF-7, using the MTT assay. GC-MS analysis showed that the main components found in the EERS were nonyl cyclopropane (4.44%), 1-tetradecane (3.66%), cyclotetradecane (2.41%), cyclododecane (1.92%), and 1-decene (1.72%). The antioxidant activity was determined through different methods. High amounts of TPC and TFC in the EERS were found. Moderate antioxidant capacity of the EERS was detected by DPPH and ABTS assays, with EC₅₀ values of 44.19 and 56.61 µg/mL and a high FRAP value of 281.79 nmol Fe²⁺ equivalent/mg extract. In the MTT assay, the EERS showed potent anticancer activity, with IC₅₀ values of 19.82, 37.001, 50.49, and 53.29 µg/mL against HT-29, HCT 116, HeLa, and MCF-7 tumour cell lines, respectively. Moreover, the results were comparable to or less potent than the standard reference drug, 5-fluorouracil. The results showed that the EERS of *Etlingera elatior* inflorescence contained a high amount of polyphenols and flavonoids, which may be the selective antiproliferative effects towards colon cancer *in vitro*.

Keywords: *Etlingera elatior*. GC-MS. MTT. Antiproliferative effect. Antioxidant activity.

INTRODUCTION

Plants are still the backbones of most medicines in several developing and under-developing countries, where people mostly depend on local herbs for curing numerous complaints. It has been estimated that around 80% of the world's population relies on plant-derived medicines for

their healthcare needs (Cragg, Newman, 2013; Ekor, 2014). In the perspective of cancer, plant-derived compounds have shown promising activities against different types of diseases. Among the various anticancer drugs used in chemotherapy practice today, a variety of these, namely camptothecin, etoposide, epipodophyllotoxin, irinotecan, paclitaxel, topotecan, vinblastine, and vincristine, are derived from plant sources, which indicates the importance of natural therapies in the treatment of cancer (Cragg, Newman, 2005).

A balance between oxidants and antioxidants is essential to maintaining good health since any alteration in this balance can lead to pathophysiological disorders,

*Correspondence: M. A. Al-Mansoub, Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia. Phone: +601136872871. E-mail: madjed_25@yahoo.fr. V. Murugaiyah, Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, Penang, Malaysia. Phone: +60124643214. E-mail: vicky@usm.my

including cancer (Hasani-Ranjbar, Larijani, Abdollahi, 2009; Saeidnia, Abdollahi, 2013). The amount of reactive oxygen species (ROS) is controlled by regulating their generation and their elimination through cellular antioxidant systems. However, under oxidative stress large amounts of ROS are produced, leading to damage to cellular proteins, lipids, and DNA, which results in the development of cancerous masses (Gupta *et al.*, 2012; He *et al.*, 2017). In recent years, plant-derived phytochemicals have gained attention as potential therapeutic agents against cancer due to their chemical diversity, global acceptability, and lack of substantial toxicity. Polyphenols and flavonoids have been acknowledged as potential anticancer agents because of their multifaceted nature, including free radical neutralising action (Batra, Sharma, 2013; Singh *et al.*, 2014).

Etilingera elatior (Jack.) is a perennial clumping plant that belongs to the family Zingiberaceae. Inflorescence of *E. elatior*, torch ginger, is an edible plant that is widely cultivated as a spice for curry in tropical and subtropical regions (Susanti *et al.*, 2013). In Malaysia, local people use young flower shoots, inflorescences, and fruits as condiments, food, medicine, and ornaments. In addition, different parts of this plant are traditionally used for the treatment of earache, wound cleansing, and to remove body odour (Mohamad *et al.*, 2005; Chan, Lim, Wong, 2011).

Many studies have been conducted to explore the phytochemical constituents of this plant. Carbohydrates, essential oils, flavonoids, saponins, terpenoids, and tannins are reported to be the main constituents of the flower, leaves, inflorescence, and rhizome of *E. elatior* (Zoghbi, Andrade, 2005; Jaafar *et al.*, 2007; Lachumy *et al.*, 2010; Ramasamy *et al.*, 2016). Numerous research groups have extensively studied the antioxidant and anticancer properties of *E. elatior*. Furthermore, *in vitro* anticancer activities of leaves, flowers, and rhizomes of *E. elatior* against HeLa (human cervical carcinoma), MCF-7 (human mammary carcinoma), MDA-MD-231 (human mammary carcinoma), CEM-SS (human T4-lymphoblastoid), HepG2 (human hepatocellular carcinoma), K-562 (chronic myelogenous leukaemia), MV-4-11 (biphenotypic B myelomonocytic leukaemia), and HT-29 (human colorectal adenocarcinoma) have been shown in various research reports (Mackeen *et al.*, 1997; Mai *et al.*, 2009; Hueh Zan *et al.*, 2011; Jusoh, Seeni, Johan, 2012), while no cytotoxic effects were observed against normal WRL-68 (human liver) and Vero (African green monkey kidney) cells. However, to

the best of our knowledge, there are no antiproliferative studies on the EERS of *E. elatior* and its antioxidant activities. Therefore, this study was designed to evaluate the selective antiproliferative effect of the EERS against four cell lines and to evaluate the antioxidant potential of *E. elatior* through free radical scavenging and ferric reducing power activities.

MATERIAL AND METHODS

Collection of plant material

Etilingera elatior (Jack.) young inflorescence was collected from Pulau Pinang (Balik Pulau), Malaysia at GPS coordinates of 5°23'06.7" N, 100°12'35.0" E. After identification by a botanist, the plant material was deposited in the herbarium unit, with a voucher specimen number of 11560, at the School of Biological Sciences, Universiti Sains Malaysia (USM).

Chemicals

Folin-Ciocalteu reagent (FCR), HPLC grade methanol, gallic acid, and L-ascorbic acid were obtained from Merck Co. (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), quercetin, 2,4,6-Tris(1-pyridyl)-5-triazine (TPTZ), 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS), and potassium persulphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate, sodium acetate trihydrate, aluminium chloride, ferric chloride hexahydrate, and ferrous sulphate heptahydrate were obtained from R & M Chemicals (Essex, UK). Hydrochloric acid, ethanol, and the other chemicals and solvents used in this study were of the highest quality available.

Extraction of plant material

The fresh sample (200 g) was cleaned, cut into small pieces, and blended with an electric grinder using 500 mL of 95% ethanol. The resultant paste was further extracted at room temperature with 1 L of 95% ethanol for 6 days with occasional shaking. Thereafter, the soaked material was filtered using Whatman filter paper, and the filtrate was allowed to settle at room temperature for 24 h, which resulted in the separation of the filtrate into two portions, i.e., the resinous sediment and the liquid fraction. The EERS was separated from the liquid fraction using filter paper and it was further dried in a hot air oven at 40 °C.

The resultant solid mass (EERS) was kept in an airtight container in the refrigerator until further experimentation. The EERS obtained was 0.78 g, and the percent yield was 0.39%. The EERS was dissolved in dimethyl sulfoxide (DMSO) for antiproliferative and antioxidant screening studies (Yoon *et al.*, 2012).

Gas chromatography/mass spectrometry (GC-MS) analysis

An Agilent gas chromatography mass spectrometer (Agilent 6890N/5973I) with electrospray ionisation was used for the phytochemical analysis. An HP-5 MS column (30 m length and 0.25 mm diameter; film thickness of 0.25 µm) was used for the separation of different phytoconstituents. The initial temperature was set to 70 °C for 2 minutes, and then it was steadily increased to 285 °C, with a 20 °C increase in each step. Helium gas was used as the carrier, with a flow rate 20 mL per minute, and 2 µL of the sample (dissolved in methanol) was injected. The transfer line was maintained at 250 °C, and the mass spectrophotometer was operated at 1717.6 EM voltage. The total run time was 60 minutes. The separated compounds were identified by comparing their retention indices and mass spectra with those in the NIST02 library. Compounds having more than 90% similarity were included in the study.

Cell cultures

MCF-7 human hormone-dependent breast carcinoma cells (ATCC® HTB-22), HeLa cervical cancer cells (ATCC® CCL-2), HT-29 colon carcinoma cells (ATCC® HTB-38), and HCT 116 colon carcinoma cells (ATCC® CCL-247) were purchased from American Type Culture Collection (ATCC) (Rockville, MD, USA). All cell lines were maintained in complete Dulbecco's Modified Eagle's Medium supplemented with 10% (vol./vol.) foetal calf serum, penicillin G (100 µg/mL), and streptomycin (100 µg/mL).

Antiproliferative/cytotoxic effect

The *in vitro* inhibitory growth effects of the EERS against a panel of human cancer cell lines, i.e., MCF-7, HeLa, HT-29, and HCT 116, were studied using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide (MTT) assay in a 96-well plate format. In this assay, 5,000–10,000 cells per well were seeded in a

96-well plate and allowed to attach for 24 h in a cell culture incubator at 37 °C with 5% CO₂. After 24 h, the cells were treated with six different concentrations of the EERS (3–100 µg/mL in DMSO) for 48 h. Media with 0.5% DMSO was used as a negative control, while 5-fluorouracil (5-FU), doxorubicin (DOX), tamoxifen (TMX), and betulinic acid (BA), at 10 µg/mL, were serially diluted and used as standard reference drugs. At the end of the treatment period, 20 µL of the MTT reagent (5 mg/mL) was added into each well of the 96-well plate and incubated for an additional 4 h in a cell culture incubator. The media was aspirated from the plate, and 100 µL of DMSO was added to each well. The absorbance of dissolved formazan crystals was measured at 570 nm, with a reference wavelength of 650 nm. The percentage of growth inhibition of the cells exposed to the treatments was calculated and compared to standard reference drugs (Revadigar *et al.*, 2017).

In vitro antioxidant activity

The EERS was standardised by determining its secondary metabolite contents (total phenolic and total flavonoid content). The antioxidant activity was evaluated through different methods, such as free-radical scavenging capacities by DPPH and ABTS and ferric-reducing antioxidant power (FRAP) activity, following well-established protocols. All antioxidant assays were performed in 96-well plate format.

Total phenolic content

For estimation of the phenolic content, Folin-Ciocalteu reagent (2 N), sodium carbonate (20%), gallic acid (0.08–10 µg/mL)/EERS (10 mg/mL), and distilled water were mixed in a ratio of 5:15:1:79 in Eppendorf tubes to a final volume of 200 µL, and then incubated at room temperature for 2 h. Later, the absorbance of the resultant mixture was measured at 765 nm, and the results are expressed as µg gallic acid (standard phenolic compound) equivalent/mg dry extract (Kumaran, Karunakaran, 2007).

Total flavonoid content

The total flavonoid content was determined by the aluminium chloride method using quercetin as a reference standard, with slight modifications (Orhan, Orhan, Ergun, 2011). In short, 100 µL of the EERS (10 mg/mL)/standard quercetin (0.39–50 µg/mL) was mixed with 20 µL of 10%

aluminium chloride, 20 μL of 1 mol/L sodium acetate, 300 μL of methanol, and 560 μL of distilled water. The reaction mixture was incubated at room temperature for 30 minutes, followed by measurement of absorbance at 415 nm. The results are expressed as μg quercetin equivalent/mg dry extract.

DPPH scavenging assay

One hundred microlitres of eight different concentrations of the EERS (0.78–100 $\mu\text{g}/\text{mL}$ in DMSO)/standard ascorbic acid (0.20–25 $\mu\text{g}/\text{mL}$) was mixed with 100 μL of DPPH reagent (200 $\mu\text{mol}/\text{L}$ in methanol), and the mixture was incubated at room temperature for 30 minutes in the dark. The absorbance of the remaining DPPH was measured at 517 nm, and the results are expressed as EC_{50} , which is the concentration of the EERS or ascorbic acid required to inhibit the formation of DPPH radicals by 50% (Al-Mansoub, Asmawi, Murugaiyah, 2014).

ABTS radical scavenging assay

The ABTS reaction mixture was prepared by mixing equal volumes of ABTS radical cation (14 mmol/L ABTS^{•+}) and potassium persulfate (4.9 mmol/L) solutions, and the mixture was allowed to react for 16–20 h in the dark at room temperature before use. The ABTS working solution was prepared by mixing 1 mL of the above solution with 40 mL of deionised distilled water to yield an absorbance equal to 0.70 ± 0.02 . The assay was performed by mixing 20 μL of eight different concentrations of the EERS (0.78–100) $\mu\text{g}/\text{mL}$ in DMSO/standard ascorbic acid (0.04–5 $\mu\text{g}/\text{mL}$) with 180 μL of the ABTS working solution in each well of a 96-well plate, and the plate was incubated for 6 min at room temperature. The absorbance of the reaction mixture was read at 734 nm, and the results are expressed as EC_{50} values (Al-Mansoub, Asmawi, Murugaiyah, 2014).

Ferric-reducing antioxidant power (FRAP) assay

The FRAP working solution was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L of FeCl_3 in a ratio of 10:1:1. For estimation of the antioxidant capacity of the EERS, 150 μL of the FRAP working solution was mixed with 50 μL of the EERS extract (10 mg/mL), followed by incubation of the reaction mixture at room temperature for

8 minutes. The absorbance was then measured at 600 nm. Ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), in the range of 1.95–250 $\mu\text{g}/\text{mL}$, was used to generate the standard curve. The antioxidant power of the EERS was estimated from the standard curve equation and is expressed as nmol Fe^{+2} equivalent/mg dry extract (Iqbal *et al.*, 2017).

Statistical Analysis

The data are presented as the mean \pm standard error of mean (SEM). The minimum inhibitory concentration (IC_{50}) and the concentration at which the scavenging activity was 50% (EC_{50}) were calculated from the linear regression equations of the dose response curve.

RESULTS AND DISCUSSION

Carcinogenesis is a multistep syndrome involving the transformation and the subsequent selective expansion of the transformed cells. Several factors, including ROS and free radicals, can initiate or contribute to cancer progression. Increased ROS levels, in turn, may cause DNA, protein, and/or lipid damage, leading to changes in chromosome instability, genetic mutation, and/or modulation of cell growth, thus leading to the development of cancer (Klaunig, Kamendulis, Hocevar, 2010). Natural antioxidants can control the levels of ROS through several mechanisms; therefore, one of the most common approaches to prevent the development of cancer is to control the generation of ROS through the use of antioxidants. Multiple studies have established the antioxidant and chemopreventive role of natural products, including flavonoids and phenolics, against a variety of cancers (Lall *et al.*, 2015). The antioxidant and anticancer properties of plant-derived phenolic compounds, such as phenolic acids and flavonoids, have been shown by various researchers (Batra, Sharma, 2013).

Chemical characterisation of the EERS by GC-MS revealed that a total of 41 compounds were present in the EERS. The GC-MS spectrum of the EERS is shown in Figure 1 (A & B). Out of these compounds, 13 compounds with a similarity index of 90 and above were included in the study. Minor compounds found to be belonging to a few classes, namely long chain aliphatic hydrocarbons, cyclic aliphatic compounds, long hydrocarbon chain, and long chain fatty acids. The retention time, the peak area percentage, molecular formula, and a molecular weight from the NIST02 library are shown in Table I.

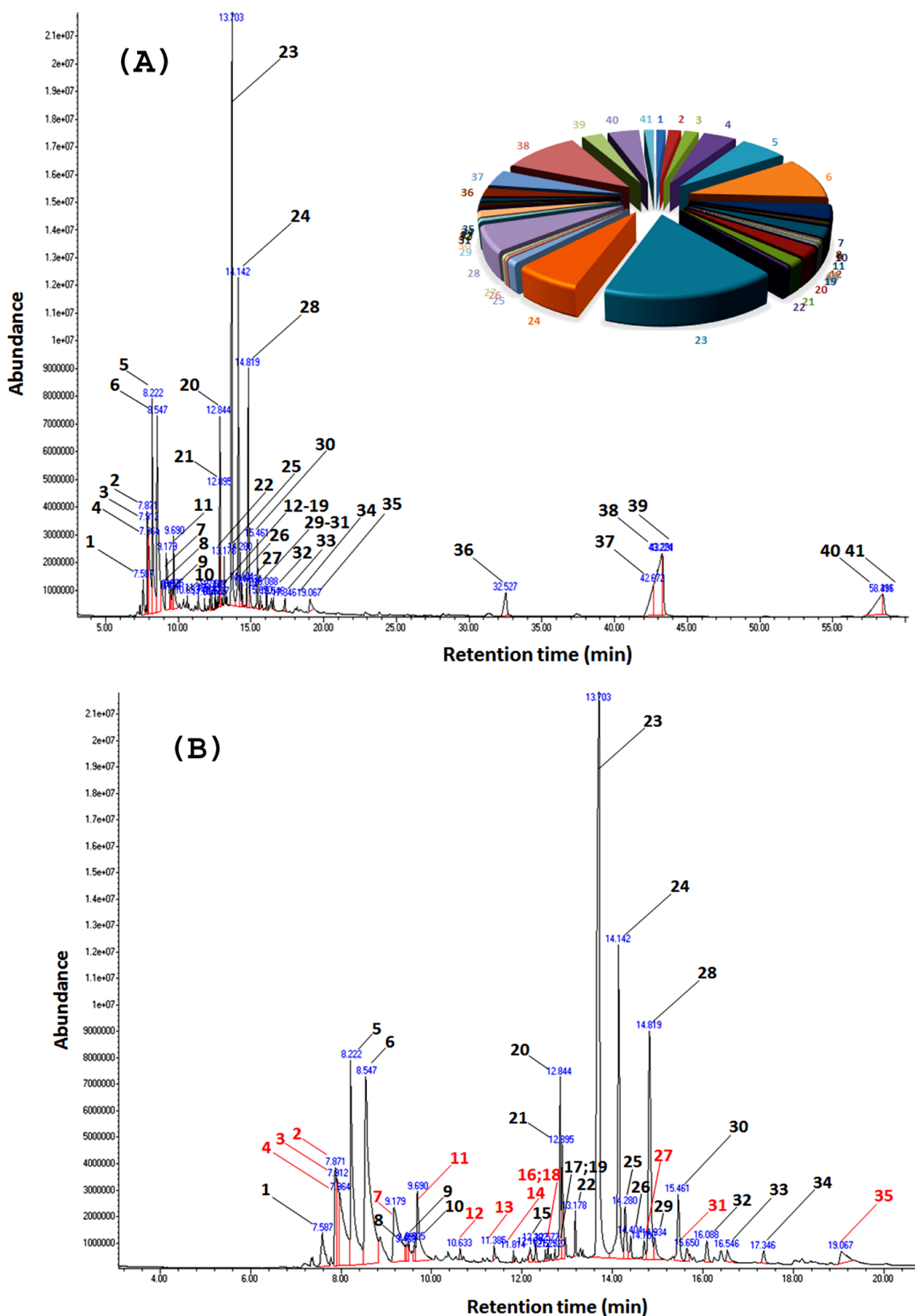


FIGURE 1 – (A) GC-MS chromatogram of EERS of *E. elatior* (Jack.) inflorescence. The pie chart illustrates the percentage of each compound (total 41 compounds) present in the extract using NIST02 library. (B) The 13 identified compounds which have similarity index 90% and above were highlighted in red colour.

TABLE I – Gas chromatography mass spectrometry (GC-MS) analysis of EERS of *E. elatior* (Jack.) inflorescence

Peak No.	Compounds	Retention time	Relative Area (%)	Molecular formula	Molecular weight
1	1-Decene	7.87	1.72	C ₁₀ H ₂₀	140.2658
2	Cyclododecane	7.91	1.92	C ₁₂ H ₂₄	168.319
3	Nonyl cyclopropane	7.97	4.44	C ₁₂ H ₂₄	168.319
4	1-Tetradecene	9.18	3.66	C ₁₄ H ₂₈	196.3721
5	Cyclotetradecane	9.69	2.41	C ₁₄ H ₂₈	196.372
6	Hexadecanoic acid, ethyl ester	10.63	0.15	C ₁₈ H ₃₆ O ₂	284.477
7	Linoleic acid ethyl ester	11.39	0.16	C ₂₀ H ₃₄ O ₂	306.483
8	Heptadecane	11.81	0.10	C ₁₇ H ₃₆	240.468
9	Hexanedioic acid, bis (2-ethyl hexyl) ester	12.30	0.26	C ₂₂ H ₄₂ O ₄	370.5665
10	Nonadecane	12.58	0.37	C ₁₉ H ₄₀	268.521
11	2-(9-octadecenyloxy)- Ethanol	14.70	0.37	C ₂₀ H ₄₀ O ₂	312.530
12	13-Tetradecen-1-ol acetate	15.65	0.31	C ₁₆ H ₃₀ O ₂	254.408
13	Beta –sitosterol	19.07	0.83	C ₂₉ H ₅₀ O	414.707

Compounds similarity index 90 and above with NIST02 library were considered for reporting.

The *in vitro* antiproliferative effects of the EERS against a panel of human cancer cell lines (HCT 116, HT-29, HeLa, and MCF-7) using the MTT assay revealed that the EERS was toxic against colon cell lines and selective towards the HT-29 cell line, with an IC₅₀ value of 19.82±2.06 µg/mL (Figure 2). The order of cytotoxicity

was HT-29 > HCT 116 > HeLa > MCF-7. Treatment of the EERS for 48 h caused no cytotoxic activity towards EA.hy926 normal human endothelial cells, with an IC₅₀ value of 69.12±0.94 µg/mL. In addition, the results were compared to the standard reference drugs 5-FU, TMX, DOX, and BA (Table II).

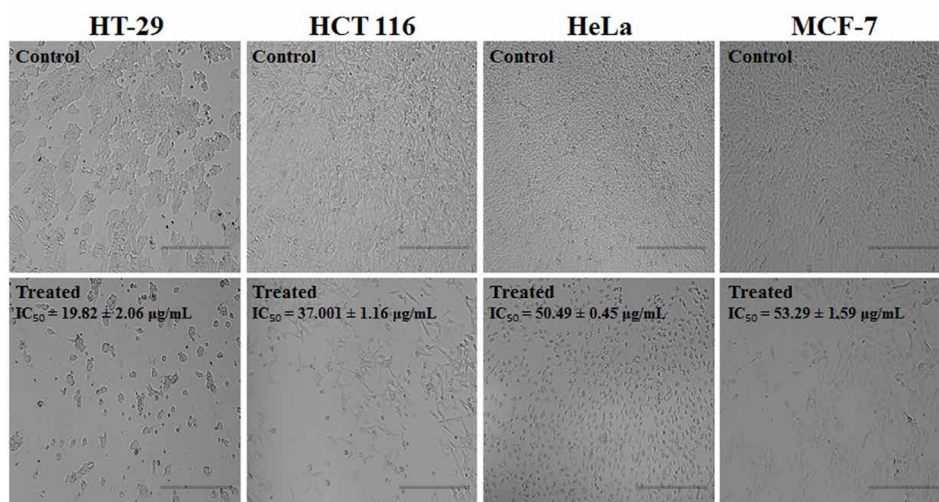


FIGURE 2 – Antiproliferative effect of EERS of *E. elatior* (Jack.) inflorescence against panel of human cancer cell lines. Photos were taken at 10× magnification (scale bar 400 µm).

TABLE II – Cytotoxic effect of EERS of *E. elatior* (Jack.) inflorescence against human cancer cell lines

Cell lines	IC ₅₀ (µg/mL)	Standard IC ₅₀ (µg/mL)
HT-29 Colon cancer	19.82 ± 2.06	20.15 ± 2.19 (5-FU)
HCT 116 Colon cancer	37.001 ± 1.16	5.00 ± 3.06 (5-FU)
HeLa Cervical cancer	50.49 ± 0.45	3.42 ± 0.05 (DOX)
MCF-7 Breast cancer	53.29 ± 1.59	8.90 ± 1.87 (TMX)
EA.hy926 Normal human endothelial	69.12 ± 0.94	8.00 ± 4.00 (BA)

Values are expressed as mean ± SEM (n=3). Where 5-FU = 5-Fluorouracil, DOX = Doxorubicin, TMX = Tamoxifen and BA= Betulinic acid are used as particular standard cytotoxic drugs.

The chemopreventive and antitumor properties of polyunsaturated fatty acids and their metabolites have been established by numerous research groups (Xu, Qian, 2014). These studies support the findings of this study, which is that the *in vitro* antiproliferative effects of the EERS might be due to polyunsaturated fatty acids and their derivatives. However, the *in vitro* cytotoxic activities of different extracts (leaves, rhizomes, stems, and flowers) of *E. elatior* against HT-29, MCF-7, and HeLa have been reported by other research groups (Habsah *et al.*, 2005; Mai *et al.*, 2009; Chan, Lim,

Wong, 2011). The current study is different from these reports in that the EERS has potent cytotoxic effects against the HT-29 cell line (IC₅₀=19.82±2.06 µg/mL), whereas these reports show comparatively moderate activity (IC₅₀=170 µg/mL). Moreover, Mackeen *et al.* (1997) reported that the ethanol extract of the young flower shoots of *E. elatior* possessed antimicrobial and cytotoxicity activity against HeLa cells. The National Cancer Institute (NCI) criterion report that a plant extract with an IC₅₀ value of <20 µg/mL is considered cytotoxic was adopted for cytotoxic characterisation

(Abdel-Hameed *et al.*, 2012). The compounds with less similarity were found in high amounts, and they were predicted to be polyphenolic compounds. Hence, we further screened the total phenolic and flavonoid contents of the EERS by well-established methods and

were found to be in a high amount of 64.95 ± 0.25 μg gallic acid equivalent/mg extract, and 20.53 ± 0.16 μg quercetin equivalent/mg extract. Table III presents the antioxidant properties and polyphenolic contents of the EERS.

TABLE III – Antioxidant activity of EERS of *E. elatior*. elatior (Jack.) inflorescence

Sample	Total phenolic (μg gallic acid equiv./mg extract)	Total flavonoid (μg quercetin equiv./mg extract)	DPPH EC_{50} ($\mu\text{g}/\text{mL}$)	ABTS EC_{50} ($\mu\text{g}/\text{mL}$)	FRAP (nmol Fe^{+2} equiv./mg extract)
EERS	64.95 ± 0.25	20.53 ± 0.16	44.19 ± 1.55	56.61 ± 0.64	281.79 ± 0.65
Vitamin C (Standard)	-	-	4.46 ± 0.20	3.27 ± 0.10	-

Values are expressed as mean \pm SEM (n = 3).

The antioxidant activity of the EERS might be mainly due to its polyphenolic contents. The antioxidant activity of flavonoids was initially reported in *E. elatior* in a study carried out by Andarwulan *et al.* (2010). The result of this study was in agreement with other studies that have been carried on methanolic extracts with similar patterns, and it reported that the phytochemical screening of (torch ginger) flowers showed the presence of flavonoids, terpenoids, saponins, tannins, and carbohydrates (Lachumy *et al.*, 2010). However, it is suggested that the phenolic content of inflorescences is attributed mainly to flavonoids, such as kaempferol and quercetin, and their derivatives. Moreover, the TPC of *E. elatior* in this study might be varied in comparison with the earlier studies due to differences in the plant parts used, the source of the plant, and the extraction methods, as reported by Ramasamy *et al.* (2016).

The antioxidant capacity of phenolic compounds is mainly attributed to their redox properties, allowing them to act as reducing agents, electron donors, oxygen quenchers, or metal chelators (Sofidiya *et al.*, 2006; Sylvie *et al.*, 2014). In a study carried by Ghasemzadeh *et al.* (2015), the authors claimed that among the three different locations in Malaysia (Kelantan, Pahang, and Johor) *E. elatior* in the northeastern region (Kelantan) exhibited potent anticancer against the tumour cell lines MCF-

7 and MDA-MB-231. It has been documented that the consumption of polyphenols may play a role in preventing many diseases, including cancer, in which free radicals are involved. Overabundant production of free radicals in the body causes damage to proteins, DNA, and RNA, and it causes the oxidation of polyunsaturated fatty acids present on the cell membrane. Thus, they cause detrimental mutations in the genome. The secondary metabolites from higher plants, especially phenolics and flavonoids, have been reported to be potent free-radical scavengers. A plethora of reports suggests that several phenolics are capable of quenching free radicals as well as the underlying mechanisms responsible for inflammation. Apart from these effects, polyphenols are also efficient at inducing several endogenous antioxidant molecules. A positive relationship between polyphenol intake and a reduced risk of some cancers has been published in some epidemiological studies (Prasad *et al.*, 2009; Xu, Qian, 2014). Besides, the antioxidant activities against these free radicals are correlated with the concentration and chemical structures of antioxidants. In the present study, the EERS was tested against a series of antioxidant models, i.e., DPPH, ABTS, and FRAP. Hence, in this study the EERS was also found to be an effective scavenger of DPPH and ABTS by donating a hydrogen atom, and it possessed excellent ferric-reducing antioxidant power,

treating the antioxidants in the sample as reductants in a redox-linked colourimetric reaction. Therefore, the overall antioxidant activity of the EERS, which has a high amount of polyphenolic and flavonoid compounds, could be associated with its anticancer activities.

The antioxidant activity measured by DPPH and ABTS radical scavenging assays was expressed as EC_{50} values, considering the fact that lower EC_{50} values suggest better free scavenging activity. The DPPH assay is a standard method used to assess the free-radical scavenging activity of phytochemical constituents. DPPH• is a stable free-radical that can accept an electron or hydrogen atom, making it a stable molecule (Wijekoon, Bhat, Karim, 2011). The ABTS assay is based on the direct production of the blue/green ABTS•+, which is generated by the oxidation of ABTS with potassium persulfate, and it is reduced in the presence of hydrogen-donating antioxidants (Floegel *et al.*, 2011). The findings of this study showed that the EERS has moderate free-radical scavenging activity in DPPH and ABTS assays, with EC_{50} values of 44.19 ± 1.55 $\mu\text{g/mL}$ and 56.61 ± 0.64 $\mu\text{g/mL}$, respectively, as compared to the reference standard of ascorbic acid. The findings of our study are consistent with a previous report, which also reported moderate antioxidant activity of *E. elatior* inflorescence (Chan, Lim, Wong, 2011). Meanwhile, the capacity toward ferric reduction was observed in FRAP, with a value of 281.79 ± 0.65 nmol Fe^{+2} equivalent/mg extract. The results are in agreement with the findings reported by Rabeta and Faraniza (2013), who found the same trend, in which methanol extractions that gave higher TPC and FRAP values compared to water extraction. Furthermore, the FRAP assay is an electron-transfer reaction, in which a ferric salt is used as an oxidising agent. This method is different from the others in that no free radicals are involved. This reaction is based on the reduction of the yellow Fe^{+3} -TPTZ complex to form the blue Fe^{+2} -TPTZ complex at low pH (Benzie, Strain, 1996). The FRAP assay is an electron transfer mechanism rather than mixed SET (single electron transfer) and HAT (hydrogen atom transfer) mechanisms. Thus, the FRAP assay in combination with other methods, it can be beneficial in distinguishing antioxidant mechanisms, as described extensively by Griffin and Bhagooli (2004). To date, there are no reports available on the antioxidant potential of the EERS of *E. elatior* (EERS) young inflorescences and its potential cytotoxicity, specifically, its prominent selectivity towards HT-29 and HeLa cell lines. Our results are significant in terms of cytotoxicity, in comparison with previous studies (Mai *et al.*,

2009; Chan, Lim, Wong, 2011). This could be due to the presence of long-chain aliphatic hydrocarbons and fatty acids. Current reports suggest that polyunsaturated fatty acids, including linoleic acid, and their metabolites are capable of acting as free-radical scavenging agents. In addition, they are capable of attenuating cyclooxygenase (COX) levels in hepatic carcinoma, and they are able to cause cell cycle arrest and apoptosis (Xu, Qian, 2014). Hence, it can be summarised that the EERS rich polyphenolic and flavonoid compounds may attribute to the antiproliferative properties towards colon, cervical, and breast cancer *in vitro*.

CONCLUSIONS

Taken together, the findings of our study show, for the first time, the *in vitro* antiproliferative effects and antioxidant properties of resinous sediment obtained from the ethanolic extract of resinous sediment of *E. elatior* young inflorescences (EERS). The EERS showed potential selective cytotoxicity against the human colorectal adenocarcinoma cell line HT-29, which indicates the potential chemopreventive role of *E. elatior* against colon cancer. Further studies on the underlying mechanism and isolation of the bioactive compounds are warranted.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in the present work.

ACKNOWLEDGEMENTS

The authors would like to thank the School of Pharmaceutical Sciences and Institute of Postgraduate Studies (IPS) at the Universiti Sains Malaysia for supporting this project through the USM Fellowship [P-DD0002/17(R)] and the Postgraduate Research Grant Scheme (PRGS) (grant no. 1001/PFARMASI/846071). All assistance in accessing the research facilities is highly appreciated.

REFERENCES

Abdel-Hameed E-SS, Bazaid SA, Shohayeb MM, El-Sayed MM, El-Wakil EA. Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of *Conocarpus erectus* L. growing in Taif, Saudi Arabia. *Eur J Med Plants*. 2012;2(2):93-112.

- Al-Mansoub MA, Asmawi MZ, Murugaiyah V. Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of *Garcinia atroviridis*: a comparative study. *J Sci Food Agric*. 2014;94(8):1552-1558.
- Andarwulan N, Batari R, Sandrasari DA, Bolling B, Wijaya H. Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chem*. 2010;121(4):1231-1235.
- Batra P, Sharma AK. Anti-cancer potential of flavonoids: recent trends and future perspectives. *3 Biotech*. 2013;3(6):439-459.
- Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239(1):70-76.
- Chan EW, Lim Y, Wong S. Phytochemistry and pharmacological properties of *Etingera elatior*: a review. *Phcog J*. 2011;3(22):6-10.
- Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta, Gen Subj*. 2013;1830(6):3670-3695.
- Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol*. 2005;100(1):72-79.
- Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014;4:177.
- Floegel A, Kim D-O, Chung S-J, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J Food Compos Anal*. 2011;24(7):1043-1048.
- Ghasemzadeh A, Jaafar HZE, Rahmat A, Ashkani S. Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of *Etingera elatior* (Jack) R.M.Sm grown in different locations of Malaysia. *BMC Complement Altern Med*. 2015;15(1):335.
- Griffin SP, Bhagooli R. Measuring antioxidant potential in corals using the FRAP assay. *J Exp Mar Bio Ecol*. 2004;302(2):201-211.
- Gupta SC, Hevia D, Patchva S, Park B, Koh W, Aggarwal BB. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. *Antioxid Redox Signal*. 2012;16(11):1295-1322.
- Habsah M, Ali AM, Lajis N, Sukari M, Yap Y, Kikuzaki H, et al. Antitumour-promoting and cytotoxic constituents of *Etingera elatior*. *Malays J Med Sci*. 2005;12(1):6.
- Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. *Inflamm Allergy Drug Targets*. (Formerly *Curr Drug Targets Inflamm Allergy*). 2009;8(1):2-10.
- He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell Physiol Biochem*. 2017;44(2):532-553.
- Hueh Zan C, Rahmat A, Akim AM, Alitheen NBM, Othman F, Lian GEC. Anti-proliferative effects of pandan leaves (*Pandanus amaryllifolius*), kantan flower (*Etingera elatior*) and turmeric leaves (*Curcuma longa*). *Food Sci Nutr*. 2011;41(4):238-241.
- Iqbal Z, Bello I, Asmawi MZ, Al-Mansoub MA, Ahmad A, Jabeen Q, et al. Vasorelaxant activities and the underlying pharmacological mechanisms of *Gynura procumbens* Merr. leaf extracts on rat thoracic aorta. *Inflammopharmacology*. 2017:1-11.
- Jaafar FM, Osman CP, Ismail NH, Awang K. Analysis of essential oils of leaves, stems, flowers and rhizomes of *Etingera elatior* (Jack) RM Smith. *MJAS*. 2007;11(1):269-273.
- Jusoh SAM, Seeni A, Johan MF. Anti-proliferative and pro-apoptotic effect of *P. sacharosa*, *E. elatior* and *P. granatum* aqueous extract on human myeloid leukaemia. *Asian J Sports Med*. 2012;1(4):146-151.
- Klaunig JE, Kamendulis LM, Hoocevar BA. Oxidative stress and oxidative damage in carcinogenesis. *Toxicol Pathol*. 2010;38(1):96-109.
- Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT-Food Sci Technol*. 2007;40(2):344-352.
- Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etingera elatior* (torch ginger) flowers. *Asian Pac J Trop Med*. 2010;3(10):769-774.
- Lall R, Syed D, Adhami V, Khan M, Mukhtar H. Dietary polyphenols in prevention and treatment of prostate cancer. *Int J Mol Sci*. 2015;16(2):3350-76.
- Mackeen M, Ali A, El-Sharkawy S, Manap M, Salleh K, Lajis N, et al. Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables (Ulam). *Int J Pharmacogn*. 1997;35(3):174-178.
- Mai CW, Wong SY, Tan EL, Balijepalli MK, Pichika MR. Antiproliferative and apoptotic studies of the standardised

- extracts of *Etlingera elatior* on human colorectal carcinoma cells. *M J Chem.* 2009;11(1):136-142.
- Mohamad H, Lajis NH, Abas F, Ali AM, Sukari MA, Kikuzaki H, et al. Antioxidative Constituents of *Etlingera elatior*. *J Nat Prod.* 2005;68(2):285-288.
- Orhan N, Orhan IE, Ergun F. Insights into cholinesterase inhibitory and antioxidant activities of five *Juniperus* species. *Food Chem Toxicol.* 2011;49(9):2305-2312.
- Prasad KN, Yang B, Dong X, Jiang G, Zhang H, Xie H, et al. Flavonoid contents and antioxidant activities from *Cinnamomum* species. *Innov Food Sci Emerg Technol.* 2009;10(4):627-632.
- Rabeta M, Faraniza RN. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*. *Int Food Res J.* 2013;20(4):1691-1696.
- Ramasamy S, Mazlan NA, Ramli NA, Rasidi WNA, Manickam S. Bioactivity and stability studies of anthocyanin-Containing extracts from *Garcinia mangostana* L. and *Etlingera elatior* Jack. *Sains Malays.* 2016;45(4):559-565.
- Revadigar V, Al-Mansoub MA, Asif M, Hamdan MR, Majid AMSA, Asmawi MZ, et al. Anti-oxidative and cytotoxic attributes of phenolic rich ethanol extract of *Musa balbisiana* Colla inflorescence. *J Appl Pharm Sci.* 2017;7(05):103-110.
- Saeidnia S, Abdollahi M. Antioxidants: Friends or foe in prevention or treatment of cancer: The debate of the century. *Toxicol Appl Pharmacol.* 2013;271(1):49-63.
- Singh B, Singh HB, Singh A, Naqvi AH, Singh BR. Dietary phytochemicals alter epigenetic events and signaling pathways for inhibition of metastasis cascade. *Cancer Metast Rev.* 2014;33(1):41-85.
- Sofidiya M, Odukoya O, Familoni O, Inya-Agha S. Free radical scavenging activity of some Nigerian medicinal plant extracts. *Pak J Biol Sci.* 2006;9(8):1438-1441.
- Susanti D, Awang NA, Qaralleh H, Sheikh Mohamed HI, Attoumani N. Antimicrobial activity and chemical composition of essential oil of Malaysian *Etlingera elatior* (Jack) RM smith flowers. *J Essent Oil Bear Pl.* 2013;16(2):294-299.
- Sylvie DD, Anatole PC, Cabral BP, Veronique PB. Comparison of *in vitro* antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa*, *Garcinia lucida* and *Hymenocardia lyrata*. *Asian Pac J Trop Biomed.* 2014;4:S625-S632.
- Wijekoon MMJO, Bhat R, Karim AA. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior* Jack.) inflorescence. *J Food Compost Anal.* 2011;24(4):615-619.
- Xu Y, Qian SY. Anti-cancer activities of ω -6 polyunsaturated fatty acids. *Biomed J.* 2014;37(3):112-119.
- Yoon MY, Oh JS, Kang H, Park J-K. Antioxidant and antibacterial behavior for sediment removed ethanol extract from sea buckthorn seed. *Korean J Chem Eng.* 2012;29(8):1069-1073.
- Zoghbi MdGB, Andrade EHA. Volatiles of the *Etlingera elatior* (Jack) R. M. Sm. and *Zingiber spectabile* Griff.: Two *Zingiberaceae* Cultivated in the Amazon. *J Essent Oil Res.* 2005;17(2):209-211.

Received for publication on 08th November 2018

Accepted for publication on 01st April 2019