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 EC\textsubscript{50} values of 44.19 and 56.61 μg/mL and a high FRAP value of 281.79 nmol Fe\textsuperscript{+2} equivalent/mg extract. In the MTT assay, the EERS showed potent anticancer activity, with IC\textsubscript{50} values of 19.82, 37.00, 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 to 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,
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, 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, 2009; Saeidnia, Abdollahi, 2013).

Etlingera 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 (Zoghi, 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-MB-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

Etlingera 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 ºC for 2 minutes, and then it was steadily increased to 285 ºC, with a 20 ºC 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 ºC, 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 ºC 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 serial 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 µg/mL in DMSO)/standard ascorbic acid (0.20–25 µg/mL) was mixed with 100 µL of DPPH reagent (200 µmol/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) µg/mL in DMSO/standard ascorbic acid (0.04–5 µg/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 (FeSO$_4$,7H$_2$O), in the range of 1.95–250 µg/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 ± 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. elatoir* (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_{16}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_{38}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 IC$_{50}$ 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 IC$_{50}$ 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).
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 (IC$_{50}$=19.82±2.06 µg/mL), whereas these reports show comparatively moderate activity (IC$_{50}$=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 IC$_{50}$ 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 (Jack.) inflorescence**

| Sample      | Total phenolic (μg gallic acid equiv./mg extract) | Total flavonoid (μg quercetin equiv./mg extract) | DPPH EC₅₀ (μg/mL) | ABTS EC₅₀ (μg/mL) | FRAP (nmol Fe²⁺ 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   | -                                               | -                                              | 4.46 ± 0.20      | 3.27 ± 0.10      | -                                |
| (Standard)  |                                                 |                                                 |                  |                  |                                  |

Values are expressed as mean ± 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,
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 μg/mL and 56.61±0.64 μg/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$^{3+}$-TPTZ equivalent/mg extract. 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 μg/mL and 56.61±0.64 μg/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$^{3+}$-TPTZ 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.

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