Sains Malaysiana 51(3)(2022): 873-882
http://doi.org/10.17576/jsm-2022-5103-21

Chemical Constituents and Antiproliferative Activity of *Eleusine indica* (L.) Gaertn.

(Kandungan Kimia dan Aktiviti Antiproliferatif *Eleusine indica* (L.) Gaertn.)

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Received: 1 January 2021/Accepted: 12 August 2021

ABSTRACT

Eleusine indica, locally known as 'rumput sambau' is widely used by traditional practitioners in many parts of the world to treat various ailments including sprained muscle, asthma, black jaundice, stomach pain, diarrhea, convulsions, and those related to infection such as malaria, influenza, dysentery as well as pneumonia. The plant has been reported to possess a wide array of biological activities, which were antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antipyretic, antiplasmodial, antiviral, hepatoprotective, and urolithiasis. Despite all the reported traditional uses and biological activities, not many chemical constituents have been isolated from the plant. In this work, *E. indica* methanolic and hexane extracts were assessed for their anti-proliferative activities against human liver cancer (HepG2) and African green monkey kidney epithelial normal (Vero) cell lines through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Although both extracts were considered to be not active on both cell lines with an IC_{50} of 91.02 ± 5.74 and 85.30 ± 3.03 μg/mL, and CC_{50} of >1000 and 639.39 ± 13.97 μg/mL, they were nevertheless more selective towards HepG2 cancer cells compared to the Vero normal cells with selectivity indexes (SI) of 10.99 and 7.50, respectively. Chemical profiling of the extracts on ultra-high performance liquid chromatography (UHPLC) coupled with a diode array detector showed a mixture of different complexity and polarities of compounds. Further isolation and purification on the hexane extract afforded β-sitosterol and stigmasterol, while the methanolic extract yielded loliolide, a benzofuran type of compound. Loliolide has been previously reported to exhibit antitumor activity, which could correlate to the higher antiproliferative selectivity against HepG2 cancer cell line of the methanolic compared to the hexane extracts. This is the first report on the isolation of β-sitosterol, stigmasterol, and loliolide from *E. indica*.

Keywords: Antiproliferative; chemical profile; *Eleusine indica;* HepG2 cell; loliolide; Vero cell

ABSTRAK

Eleusine indica, atau nama tempatannya 'rumput sambau' digunakan secara meluas oleh pengamal perubatan tradisi di kebanyakan negara untuk merawat pelbagai jenis penyakit termasuk terseliuh otot, asma, penyakit kuning, sakit perut, cirit-birit, sawan dan yang berkaitan dengan jangkitan seperti malaria, influenza, disentri serta radang paru-paru, termasuklah Malaysia. Dalam kajian ini, ekstrak metanol dan heksana *E. indica* dinilai untuk aktiviti anti-proliferatifnya terhadap sel tunggal barah hati manusia (HepG2) dan sel tunggal ginjal monyet hijau Afrika (Vero) melalui asai 3-(4,5-dimetiltiazol-2-yl)-2,5-difenil tetrazolium bromide (MTT). Walaupun kedua-dua ekstrak dianggap tidak aktif pada kedua-dua jenis sel tunggal dengan nilai masing-masing IC_{50} 91.02 ± 5.74 dan 85.30 ± 3.03 μg/mL, dan CC_{50} > 1000 dan 639.39 ± 13.97 μg/mL, namun mereka lebih selektif terhadap sel kanser HepG2 dibandingkan sel normal Vero dengan indeks selektiviti (SI) masing-masing 10.99 dan 7.50. Profil kimia ekstrak pada kromatografi cecair berprestasi tinggi (UHPLC) yang dilengkapi dengan alat pengesan dioda menunjukkan campuran kerumitan dan polariti sebatian yang berbeza. Kerja pengasingan dan pemurnian lebih lanjut pada ekstrak heksana menghasilkan β-sitosterol dan stigmasterol, sementara ekstrak metanol menghasilkan loliolida iaitu sejenis sebatian benzofuran. Loliolida sebelumnya dilaporkan menunjukkan aktiviti antitumor, yang mungkin boleh dikaitkan dengan antiproliferatif selektiviti terhadap sel tunggal barah HepG2 yang lebih tinggi oleh ekstrak metanol dibandingkan dengan ekstrak heksana. Ini adalah laporan pertama mengenai pemencilan β-sitosterol, stigmasterol dan loliolida daripada *E. indica*.

Kata kunci: Antiproliferatif; *Eleusine indica;* loliolida; profil kimia; sel tunggal HepG2; sel tunggal Vero

Introduction

Eleusine indica, locally known as Goosegrass or 'rumput sambau' is a perennial herb that belongs to the Poaceae family and can be found in the tropical region. This species of *Eleusine* has been used as traditional medicine in different parts of the world to treat various illnesses such as bladder disorder, relieve pain caused by straining abdominal muscles, children's convulsion, lung infections, retention of urine and oliguria, and hypertension (Abdul et al. 2015; Alamgir et al. 2008). In India, some parts of the plant such as the roots and the seeds are used as food and can be eaten raw or cooked. The young seedling is also used as a side dish with rice and the seeds are sometimes used as famine food (Abdul et al. 2015).

In Peninsular Malaysia, the leaves of the plant are traditionally pounded to extract its juice to hasten the delivery of placenta for women, and infusion of the leaves has also been used to help ease vaginal bleeding. The whole plant could be utilized to treat inflammatory diseases by using it either dried or fresh and the roots are useful in treating asthma by drinking its decoction (Abdul et al. 2015). People of Kadazan-Dusun in Sabah utilized roots mixture of *E. indica* and *Capsicum* sp. (Solanacae) that been boiled to treat haemorrhoids (Kulip et al. 2000). An aqueous extract obtained from the infusion of *E. indica* aerial part with rice has also been consumed by the local people of Kadazan-Dusun in Sabah to treat symptoms related to flu (Piah 2020).

Biological investigations on *E. indica* have shown that this plant possesses antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antipyretic, antiplasmodial, antiviral, hepatoprotective, and urolithiasis activities (Abdul et al. 2015; Alamgir et al. 2008; Al-Zubairi et al. 2011; Desai 2017; Iberahim et al. 2015; Iqbal & Gnanaraj 2012; Lim 2016; Morah & Otuk 2015). Despite all these reported traditional uses, and pharmacological activities, not many phytochemicals have been isolated from the plant. Early in 1978, flavonoid patterns of the genus *Eleusine* been reported which includes orientin, vitexin, isovitexin, saponarin, tricin, isoorientin, violanthin, and lucenin - 1 (Hilu et al. 1978). However, only the former five flavonoids were constituted in *E. indica*. Phytochemical screening on the plant methanolic and hexane extracts showed the presence of tannins, flavonoids, triterpenoids, alkaloids, steroids, quinones, and phenols (Iberahim et al. 2015). Hitherto, only a few phytochemicals have been isolated from the plant which includes sterol glucosides, 3-O-β-D-glucopyranosyl-β-sitosterol, and its 6'-O-palmitoyl derivatives, and the flavonoids schaftoside, vitexin, and isovitexin (Desai 2017; Iqbal & Gnanaraj 2012; Phuong et al. 1994). A recent metabolite fingerprinting and profiling on the plant extract has characterized two more phytochemicals known as p-coumaric acid and isoschaftoside along with a series of primary metabolites and amino acids (Peñaloza et al. 2018).

E. indica has been reported to be safe on the African green monkey kidney epithelial normal (Vero) cell line (Iberahim et al. 2015). However, to the best of our knowledge, the plant has not been evaluated against human liver cancer (HepG2) cell lines. Therefore, the present study explored more on the antiproliferative activity of the hexane and methanol extracts of *E. indica* against HepG2 and Vero cell lines to obtain the selectivity index (SI) value in further ensuring the safety of the extracts. Comparative information on the chemical composition of the extracts and structural complexity were also obtained through profiling on reversed-phase ultrahigh-performance liquid chromatography (UHPLC). In addition, a first report on the isolation of a benzofuran type compound, loliolide, from the methanolic extract and common sterols, β-sitosterol, and stigmasterol from the hexane extract of *E. indica* were also included.

Materials and Methods

Chemicals and Reagents

Methanol (MeOH), hexane (Hex), and acetone (Ace) of analytical grade. MeOH and acetonitrile (MeCN) HPLC grade were purchased from RCI Labscan (Bangkok, Thailand) and ultra-pure water (UPW) was from Sartorius. *E. indica* was collected on 14th January 2018 in Tanjung Karang, Selangor, Malaysia. A voucher specimen DBKL 177 was identified by a certified botanist, En. Ahmad Zainudin Ibrahim and deposited at the Herbarium Taman Botani Perdana Kuala Lumpur.

Sample Processing and Extraction

The plant material (10 kg) was cut into small pieces and dried in the oven at 40 ºC. The dried sample was weighed and ground. Then, the maceration technique was applied in the extraction procedure whereby the ground sample was extracted successively using n-Hex and MeOH at room temperature for 72 h. The extracts were filtered, and the solvents were evaporated under reduced pressure affording 10.55 g of hexane and 41.62 g of methanolic extracts. The extracts were stored at 4 ºC before being further analyzed.

HPLC EQUIPMENT

The HPLC analyses were performed using the DIONEX Ultimate 3000 HPLC system (ThermoFisher, USA) coupled with a photodiode array detector (PDA) and equipped with an auto-sampler injector. In addition, for the semi-preparative system, a fraction collector and 10 mL sample loop were also included. Chromatography was performed on Hypersil GOLD C18 column (Thermo Scientific, Malaysia) with a pore size of 175 A, and dimension (250 mm × 4.6 mm, i.d., 5 µm) for analytical UHPLC and (250 mm × 10 mm, i.d., 5µm) for semi-preparative HPLC. The control of the instrument and the data analyses were conducted by software Chromeleon version 7.2 provided by the supplier. Recycling HPLC was performed on JAI, model LC-9103 (Japan Analytical Industry Co., Ltd.) equipped with reciprocating double plunger pump type P-9140B and UV detector of wavelength set to 210 nm. The separation was carried out on a preparative column JAIGEL-ODS-AP, SP-120-15 (20 mm × 250 mm).

UHPLC CHEMICAL PROFILING OF *E. indica*

Two mg/mL of the extracts were weighed precisely and dissolved in methanol and were filtered through a 0.45 µm PTFE filter (Agilent) into 2 mL screw cap vials prior to UHPLC analysis. Chromatographic conditions were optimized in order to reach baseline peaks separation for the overall observable chemical components. For this purpose, different mobile phases with varying gradients were employed. The mobile phase consisting of MeCN/UPW with linear and curve gradients set up delivered good baseline separation for the hexane and methanolic extracts, respectively. Prior to injection, the needle was washed three times, with 100% MeCN. For the hexane extract, an initial gradient was set at 10% MeCN: 90% UPW and gradually increased to 95% MeCN: 5% UPW at a duration of 24 min. Whereas for the methanolic extract, the mobile phase was initially set at 10% MeCN: 90% UPW and gradually increased with curve 7 to 100% MeCN: 0% UPW at a duration of 20 min. The column conditioning and equilibrium were performed in 5 min attaining the initial condition. A 10 µL of the filtered extracts were injected into the chromatographic column thermostatically maintained at 30 °C. The flow rate used was 1.00 mL/min and the detection of the eluted peaks was performed at 210 nm.

ISOLATION AND PURIFICATION OF *E. indica* CHEMICAL CONSTITUENTS GENERAL EXPERIMENTAL PROCEDURE

^{1}H and ^{13}C NMR spectra were recorded in suitable deuterated solvent on a Bruker 600 Ultrashield NMR spectrometer measured at 600 and 150 MHz, respectively. Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were performed using pre-coated, aluminum-backed, silica gel 60 F_{254} (0.2 mm thickness) and glass supported silica gel 60 F_{254} (0.5 and 1.0 mm thickness), respectively. The sample was spotted on the TLC plates using a piece of fine glass capillary tube and then developed in a saturated chromatographic tank at room temperature. Vacuum liquid chromatography (VLC) was performed with silica gel 60, 70-230 mesh ASTM (Merck 7734), whereas column chromatography (CC) was carried out using either cross-linked dextran Sephadex LH-20 gel or silica gel 60, 70-230 mesh ASTM (Merck 7734) where applicable. The ratio of the gel to the sample used was approximately 30:1. The gel was made into slurry by addition of desired organic solvent and packed into a glass column, and allowed to equilibrate for at least an hour prior to use. Spots and bands for compounds on TLC and PTLC were detected using UV light (254 and 365 nm).

ISOLATION OF Β-SITOSTEROL AND STIGMASTEROL FROM HEXANE EXTRACT

The hexane extract (10 g) of the *E. indica* was initially fractionated with CC packed with Sephadex LH-20. The solvent system used was MeOH employing isocratic elution with TLC monitoring to afford 10 fractions. Fraction 4 was observed to contain promising compounds, thus subjected to CC packed with silica gel and eluted with Hex and Ace in an increasing polarity manner. Of the obtained 15 fractions, fraction 9 (15 mg) was further chromatographed on PTLC using solvent system Hex: Ace (8:2) in multiple development techniques to isolate β-sitosterol (8 mg) and stigmasterol (7 mg).

ISOLATION OF LOLIOLIDE FROM METHANOLIC EXTRACT

A 40 g of *E. indica* methanolic extract was initially subjected to VLC to afford 25 fractions. Of these, fraction 18 was chosen for further isolation and purification procedures using semi-preparative HPLC, followed by recycling HPLC. The details of the procedures are explained below.

SEMI-PREPARATIVE HPLC

Eight mg/mL of the extracts were weighed precisely and dissolved in methanol and were filtered through a 0.45 µm PTFE filter (Agilent) into 50 mL screw cap vials prior to semi-preparative HPLC analysis. The mobile phase

consisted of UPW (A) and MeCN (B). A gradient elution program was used as follows: 10 - 95% B (0.00 - 18 min), 95% B (18 - 24 min) and 95-10% B (25 - 30 min). The absorbance was monitored at 210 nm. A 300 µL sample in each injection was introduced into the system at 30 °C and a flow rate of 4.7 mL/min afforded 36.5 mg component of interest which was then further purified by recycling HPLC.

RECYCLING-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The partially purified component (36.5 mg) was dissolved in 10 mL of 80% MeCN: 20% UPW and injected into the recycling HPLC system. The separation was performed with an isocratic elution of 80% MeCN: 20% UPW. The flow rate of the system was set at 4 mL/min and the absorbance was set to 210 nm. Thirty min was allocated to condition the column and to monitor the baseline. After four complete cycles, loliolide (10 mg) was eluted at minute 242th.

ANTIPROLIFERATIVE ACTIVITY OF *E. indica* EXTRACTS CELL LINES CULTURE

HepG2 cell was acquired from Dr. Normala at AuRIns, UiTM, while Vero cell was obtained from Malaysia Institute of Pharmaceutical & Nutraceuticals (IPharm). Both cell lines were cultured in a growth medium containing Dulbecco Minimal Essential Media (Sigma) supplemented with 10% (v/v) Fetal Bovine Serum (Sigma) and 1% of 1000 µg/mL of Penicillin/Streptomycin (Nacalai Tesque). Incubation was carried out at 37 °C with an atmospheric 5% (v/v) CO_2 to allow the growth of the cells. Growth media were changed every 2 days and subculture was carried out when 80% cell growth confluency was reached.

MTT ASSAY

The MTT assay was carried out as reported by Azmi et al. (2020). Briefly, 100 µL of 150,000 cells/mL suspension was added into a 96-wellplate. The plates were incubated overnight in an incubator supplemented with 5% (v/v) CO_2 at 37 ºC to allow cells to be attached. The next day, the media was removed from the wells. The highest concentration wells were added with 150 µL of fresh growth media containing the highest concentration of drug whereas the other wells were added with 100 µL of fresh growth media.

Serial dilutions of a 2-fold factor covering a range from 1000 - 15.625 µg/mL were performed whereby 50 µL solution was removed from the highest concentration wells into the next column and mixed appropriately. This step was repeated until the last column and from this column, 50 µL aspirated was discarded. Then, 100 µL of media with 1% (v/v) DMSO were added into the wells which served as negative control while other wells served as positive control without drugs. Plates were served in triplicate. The plates were incubated at 37 °C for 24 h supplemented with 5% (v/v) CO_2. After incubation, the test plates were inspected under an inverted microscope (Nikon, Japan) to observe the normal growth of both cells. Additional information was recorded, such as drug insolubility or contamination. All media were discarded from the wells. Finally, 50 µL of 1 mg/mL MTT solution was added to each test plate and incubated for 3 to 4 additional hours (until a subtle colour change was observed, but the maximum hour taken was not more than 4 hours).

The cell absorbance from the assay plate (Figure 1) was measured by using a microplate reader (Tecan, USA) at a wavelength of 570 nm. The percentage growth inhibition was calculated using the formula:

$$Cell\ viability\ (\%) = \frac{Absorbance\ value\ of\ test\ compound}{Absorbance\ value\ of\ Negative\ Control} \times 100$$

STATISTICAL ANALYSIS

Statistical Analysis was performed by SPSS 23.0 using one-way ANOVA. All the results were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

ANTIPROLIFERATIVE ACTIVITY OF *E. indica* CRUDE EXTRACTS

Antiproliferative activity of the methanolic and hexane extracts of *E. indica* is shown in Table 1. Both methanol and hexane extracts were found to be non-toxic on the HepG2 cancer cell line with IC_{50} values of 91.02 ± 5.74 µg/mL and 85.30 ± 3.03 µg/mL, respectively. Similarly, very high concentrations of the extracts were required to achieve 50% cell viability (CC_{50}) for the Vero normal cell line which was more than 1000 µg/mL for methanol extract and 639.39 ± 13.97 µg/mL for the hexane extract indicating the non-toxic property. These are drawn based on the guidelines given by the American National Cancer Institute whereby in order for an extract to be considered active, its concentration to achieve 50% cell proliferation inhibition (IC_{50}) after an exposure time of 24 h should be less than 30 µg/mL (Suffness et al. 1990).

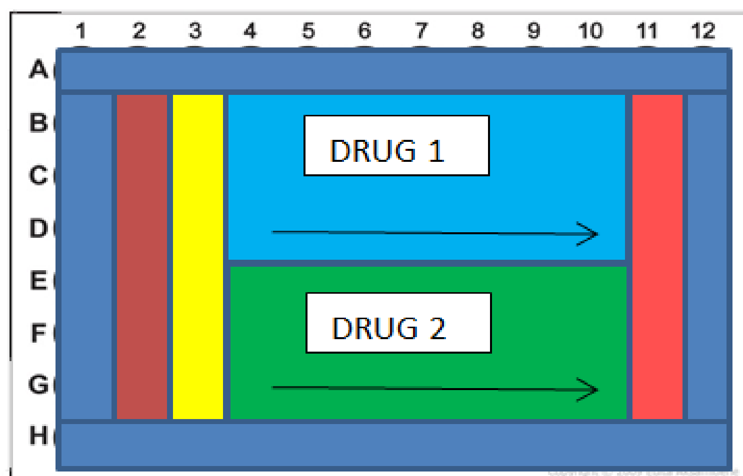

FIGURE 1. Assay plate

Nevertheless, the methanolic and hexane extracts were found to be more active against the cancer cells compared to the normal cells with selectivity indexes (SI) of 10.99 and 7.50, respectively. These SI are considered good as a value greater than 2 is indicating high selectivity of the extracts in inducing toxicity towards the cancer cells rather than normal cells (Artun et al. 2016; de Oliveira et al. 2015). Previous cytotoxicity screening on the methanolic and hexane extracts of *E. indica* against Vero cell line reported IC_{50} of 2.07 and 5.62 mg/mL, respectively, way higher than the present work (Iberahim et al. 2015). This might be due to the variation in the chemical composition of the extracts which could be explained based on plant geographical and seasonal collections (Ahmad & Salim 2015). The *E. indica* sample studied by the above group was collected in Semenyih around April 2012, whereas ours was from Tanjung Karang collected in January 2018, both Malaysia.

TABLE 1. Antiproliferative activity of *E. indica* extracts expressed in IC_{50}, CC_{50}, and SI

Extracts	HepG2 IC_{50} (µg/mL) ± SD	Vero CC_{50} (µg/mL) ± SD	Selectivity Index (SI) (CC_{50} Vero/IC_{50} HepG2)
Methanol	$91.02 \pm 5.74^{**}$	>1000	>10.99
Hexane	$85.30 \pm 3.03^{**}$	$639.39 \pm 13.97^{**}$	7.50

CC_{50} is the concentration at which 50% of cells survive and IC_{50} is the concentration at which 50% of cells death occurs. Statistical analysis was conducted using one-way ANOVA ($p<0.0001$) where ** denotes a statistically significant difference when between the extracts

UHPLC CHEMICAL PROFILING OF *E. indica* EXTRACTS

Chromatographic profiles of crude extracts can be obtained through UHPLC. Information on the chromatogram can be used to relatively compare the chemical composition and the molecular structure complexity in the extract. As shown in Figure 2, the chromatographic profile of both methanolic and hexane crude extracts of *E. indica* acquired at UV wavelength 210 nm were unalike. The hexane extract (top panel) portrayed quite a distinctive polarity of major constituents shown by the peaks that appeared at min 10.50 and min 21.00 to 24.00. In addition, minor constituents of high and medium polarities were also observed at the left and middle parts of the chromatogram, respectively. Chromatogram of the methanolic extract (low panel) contains major polar components which appear at min 2.00 - 3.70 min and a medium polar component at min 11.80. On the other hand, the minor components of the extract exhibit diverse mixture polarities indicated by the small peaks eluted within min 7.50 - 25.00.

Qualitative analysis on the chromatogram of the extracts is complemented with a thorough literature search on the classes of compounds reported from the species *Eleusine indica* and the genus *Eleusine* that could exhibit UV absorption at the acquired wavelength of 210 nm. Thus far, sterol glucosides, flavonoids, phenolic, amino acids, and a series of primary metabolites have been reported from *E. indica* (Desai 2017; Iberahim et al. 2015; Phuong et al. 1994). On the other hand, the genus *Eleusine* has been constituting compounds from the classes' hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids (Chandrasekara & Shahidi 2015). All of these classes of compounds exhibit diverse molecular polarities, supporting the observed chromatographic profile of the extracts in this work. Furthermore, the reported classes of compound contain benzoic chromophore and auxochromes that exhibiting strong π-π* and n-π* electronic transitions, respectively, at 210 nm UV region (Goldfarb et al. 1951; Salim et al. 2013; Zhang et al. 2016).

ISOLATION AND PURIFICATION OF *E. indica* CHEMICAL CONSTITUENTS

Based on the chromatographic profiles in Figure 2, the hexane extract showed more distinctive major constituents compared to the methanolic extract. Thus, conventional chromatographic methods were applied to fractionate and purify some promising constituents. Initially, the extract was fractionated through CC packed with Sephadex LH-20 employing isocratic elution of solvent MeOH and the eluents were TLC monitored. This practice is to ensure the removal of higher molecular weight molecules particularly chlorophyll that hinder the purification compounds of interest. The promising fraction was further subjected to repeated CC and

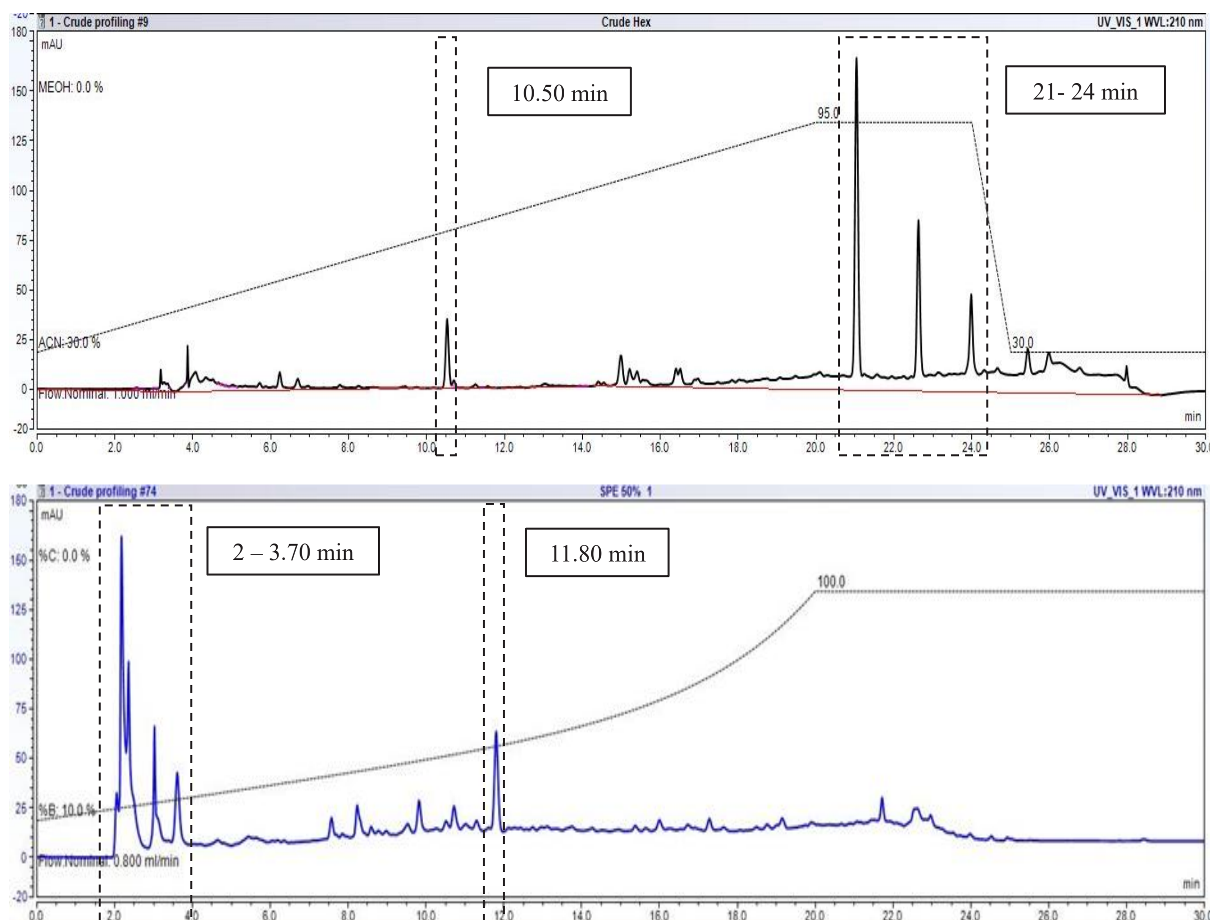

FIGURE 2. HPLC chromatograms of *E. indica* Hexane (top panel) and Methanolic (low panel) extracts

multiple development PTLC methods employing solvent system Hex and Ace in varying polarities finally isolated two compounds. The NMR structural elucidation showed that the compounds are stigmasterol and β-sitosterol, and it was confirmed with the literature values (Kamal et al. 2016; Salim 2013). Although both compounds are commonly found in the plant, this is the first report on the isolates from *E. indica*.

For the methanolic extract, due to the diverse polarities of the compound, it was subjected to various chromatographic techniques including vacuum liquid chromatography (VLC) and semi-prep HPLC to fractionate the extract and finally r-HPLC for purifying the promising constituent. Figure 3 shows the chromatographic cycles in the separation process of the promising constituent detected 210 nm wavelength. On sample injection, the eluent was drained before minute 52 to remove the impurities. When the desired peaks appeared at minute 52, the valve was then switched for the eluent to be channeled back to the column. After the desired peaks were completely eluted, the eluent was drained again to remove the remaining impurities. As shown in the r-HPLC chromatogram, this practice was done four times to ensure that the fourth cycle only contains the compound of interest. The whole purification process took four cycles to achieve a good baseline resolution for the peak whereby the peak of interest was eluted at min 242nd. Each cycle took 60 min and the whole complete cycle took 254 min to finish.

Structural elucidation of the isolated compound through NMR data analysis and comparison with literature value (Yang et al. 2011; Yuan et al. 2018) confirmed it to be a benzofuran type compound known as loliolide. Loliolide was previously reported as a constituent of other plants including *L. salicaria, H. angiospermum*, *A. lappa*, *S. oleraceus, P. campanulatus* (Cav.), *P. indicus, M. alba*, and *M. whitei* (Grabarczyk et al. 2015). Similar to stigmasterol and β-sitosterol, this is also a first report on the isolation of loliolide from *E. indica*. Loliolide has been shown to exhibit various biological activities, such as antitumor and antimicrobial activities (Murata et al. 2019). This could correlate to the higher antiproliferative selectivity against HepG2 cancer cell line of the methanolic compared to the hexane extracts. The ^{1}H and ^{13}C NMR data of stigmasterol, β-sitosterol, and loliolide are presented in Table 2, while their molecular structures are shown in Figure 4 accordingly.

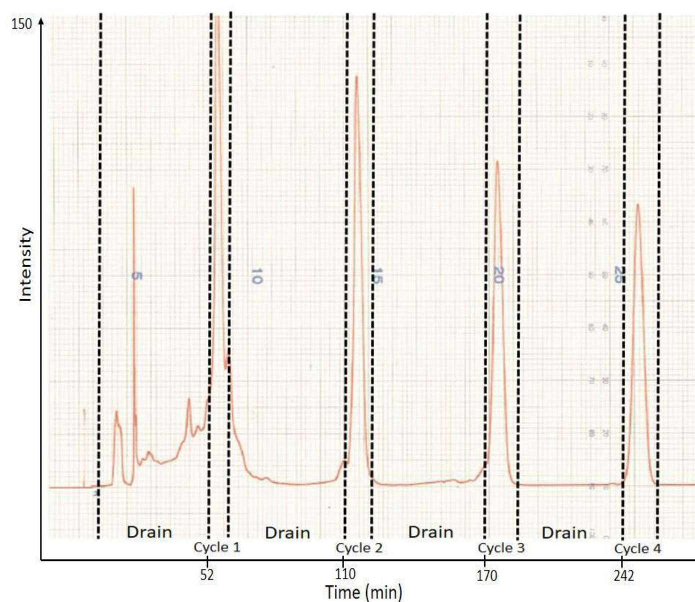

FIGURE 3. Recycling HPLC chromatogram of loliolide

TABLE 2. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) data of Stigmasterol[a], and β-Sitosterol[a] ($CDCl_3$, in ppm) (Salim et al. 2013) and lolilolide[b] (MeOD, in ppm) (Yang et al. 2011) isolated from *E. indica*

Position	Stigmasterol[a]		β-Sitosterol[a]		Position	Loliolide[b]	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)		δ_H (ppm)	δ_C (ppm)
1	α:1.45-1.40 β: 1.13	37.33	α: 1.35-1.30 β: 1.25-1.00	37.30	1	-	35.73
2	α: 1.55-1.50 β: 1.35-1.30	31.63	α: 1.55-1.45 β: 1.35-1.30	31.60	2	α: 1.97 β: 1.53	49.53
3	3.54	71.76	3.53	71.76	3	4.33	66.1
4	2.28-2.20	42.20	2.24 2.00	42.26	4	α: 2.46 β: 1.78	46.99
5	-	140.79	-	140.69	5	-	87.8
6	5.35	121.74	5.36	121.73	6	-	170.1
7	α: 2.28-2.20 β: 1.80-1.60	31.95	α: 2.00 β: 1.75-1.60	31.93	7	5.69	112.2
8	1.45-1.40	31.95	1.25-1.00	31.93	8	-	181.5
9	1.45-1.40	50.13	1.44-1.40	50.15	9	1.47	25.8
10	-	36.53	-	36.50	10	1.27	26.3
11	α: 1.55-1.50 β: 1.35-1.30	21.12	α: 1.55-1.45 β: 1.25-1.00	21.08	11	1.78	29.8
12	α: 1.55-1.50 β: 1.35-1.30	39.75	α: 1.55-1.45 β: 1.35-1.30	39.79			
13	-	42.34	-	42.29			
14	1.45-1.40	56.77	1.44-1.40	56.75			
15	α: 1.80-1.60 β: 1.35-1.30	24.30	α: 1.75-1.60 β: 1.35-1.30	24.36			
16	α: 1.80-1.60 β: 1.35-1.30	28.89	α: 1.75-1.60 β: 1.35-1.30	28.99			
17	1.55-1.50	56.79	1.55-1.45	56.83			
18	0.69	11.99	0.69	12.00			
19	1.02	19.40	1.02	19.41			
20	2.28-2.20	40.30	1.75-1.60	36.14			
21	0.92	21.09	0.93	18.78			
22	5.04	138.31	α: 1.20 β: 1.25-1.00	33.95			
23	5.16	129.30	α: 1.40 β: 1.25-1.00	26.06			
24	2.28-2.20	51.22	1.55-1.45	45.81			
25	1.80-1.60	31.93	1.83	29.15			
26	0.81	21.10	0.81	19.84			
27	0.84	19.04	0.83	19.04			
28	1.45-1.40	26.12	1.55-1.45	23.10			
29	0.86	12.24	0.87	11.99			

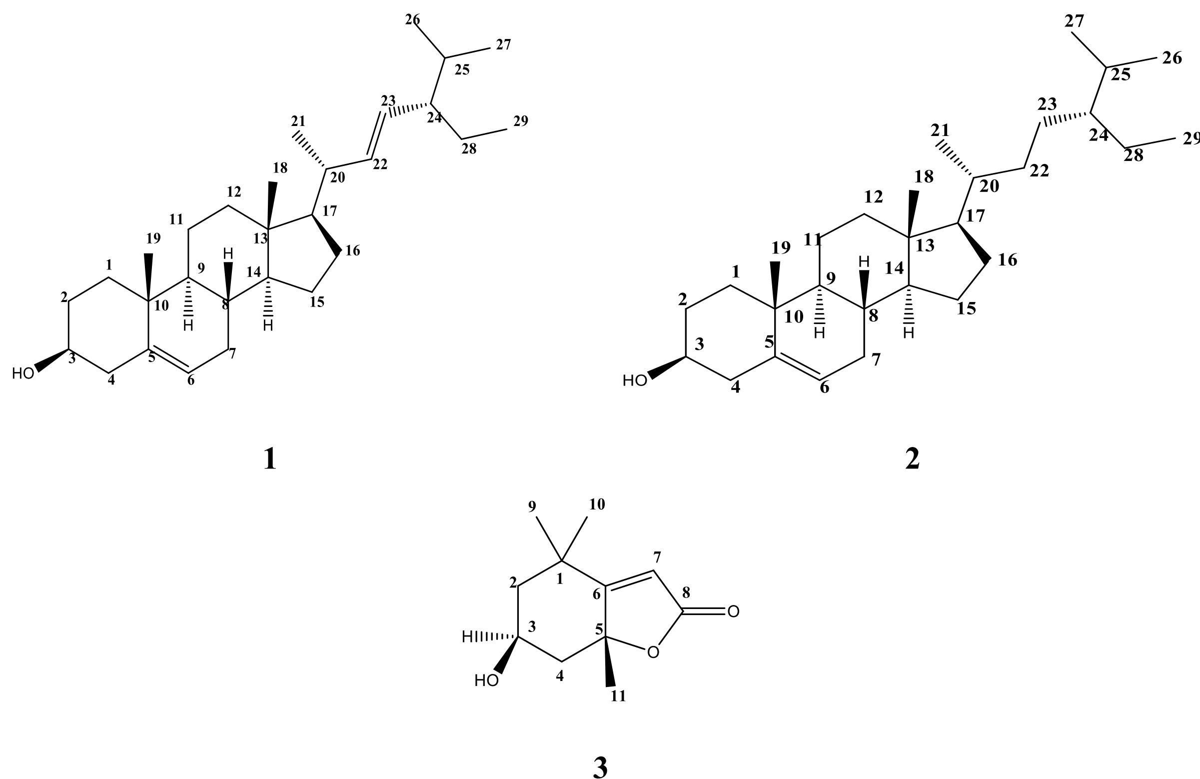

FIGURE 4. Structures of the isolated chemical constituents of *E. indica*: Stigmasterol (**1**), β-Sitosterol (**2**), and loliolide (**3**)

CONCLUSION

In this study, both methanolic and hexane extracts of *E. indica* were considered to be not active but were more selective towards the HepG2 cancer cells compared to the Vero normal cells. The chromatograms of both hexane and methanolic extracts showed different polarities of chemical constituents where conventional chromatographic technique on the hexane extract afforded stigmasterol and β-sitosterol. Whereas modern chromatographic methods applied on the methanolic extract successfully isolated a benzofuran type compound named loliolide. Loliolide has been previously reported to exhibit antitumor activity, which could correlate to the higher antiproliferative selectivity against HepG2 cancer cell line of the methanolic compared to the hexane extracts. Stigmasterol, β-sitosterol, and loliolide are the first time reported from *E. indica*. Further phytochemicals work should be done on the plant as chemical composition usually varies based on its geographical location and seasonal collections.

ACKNOWLEDGEMENTS

The authors would like to acknowledge (1) Institute of Research Management and Innovation (IRMI), Universiti Teknologi MARA for funding the study (IRMI grant no. 600-IRMI 5/3/GIP (032/2019)); (2) Faculty of Applied Science, and (3) Atta-ur-Rahman Institute for Natural Product and Discovery (AuRIns), Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia for providing facilities throughout this study.

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