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Research Article

CYTOTOXICITY ACTIVITY OF *CHRISTIA VESPERTILIONIS* LEAVES EXTRACTS AGAINST HELA CELLS AND THE DETECTION OF COMPOUNDS BY GC-MS

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ABSTRACT

Plants especially herbs have been utilized by old society for some therapeutic effects prior. In the present day, the notoriety of utilizing plants particularly for anticancer activity has continued expanding. Numerous examinations have been directed to separate out all phytochemical compounds contained in the herb that may be responsible of remedial impacts, for example, anticancer activity. In this research, *Christia vespertilionis* leaves extracts have been utilized to determine the cytotoxic activities of the plant extract towards human cervical cancer cell line (HeLa). *C. vespertilionis* leaves have been fractioned utilizing partitioned extraction from crude methanol extract followed by partitioning extract with increasing polarity namely hexane, dichloromethane (DCM), chloroform, n-butanol and aqueous fractions. Based on the discoveries, DCM extract of *C. vespertilionis* leaves has demonstrated the most elevated cytotoxicity activities towards HeLa cell line shown significantly by lowest half inhibitory concentration (IC_{50}). DCM extract displayed highest cytotoxicity with IC_{50} estimation of 63.68, 55.42 and 53.04 µg/mL at 24, 48 and 72 hours respectively. Extensive cell detachment and the presence of apoptotic bodies in 24 hours may propose cytotoxicity activity of the extract. Additionally, the presence of various phytochemical compounds in the extract utilizing gas chromatography-mass spectrometry investigation, for example, 2-propenoic acid, hexadecanoic acid, cyclododecane,9,12,15-octadecatrienoic acid, and phytol may be the explanation behind the cytotoxic activities displayed by the extract onto the HeLa cell lines. It was recommended that this plant may have pharmacological significance in the future.

Keywords: *Christia vespertilionis*; Cytotoxicity; GC-MS; HeLa cell line; SRB

INTRODUCTION

Christia vespertilionis (L.f.) Bakh.f[1], which is otherwise called Daun Rerama in Malaysia[2] was categorized under Fabaceae family. It is broadly found in tropical Southeast Asia regions and is local to China, Cambodia, Indonesia, Vietnam, Thailand and Malaysia[3]. It has been utilized in numerous alternative and complimentary medicines and its extracts had been utilized to treat snake bite, tuberculosis, bone fracture, blood flow problems, bronchitis and fevers[3,4]. *C. vespertilionis* is pharmacologically dynamic due to the presence of various phytochemicals. Alkaloids, alkanes, triterpenes, unsaturated fats, phenols and long chain alcohols have been recognized as principle constituents in this plant[5,6].

Figure 1: *Christia vespertilionis* plants can be found abundantly in Malaysia and has been used widely as medical plants among Malay people

C. vespertilionis is picking up notoriety in Malaysia as it has exhibited various therapeutic effects including anticancer properties. Past examinations demonstrated that *C. vespertilionis* can possibly repress multiplication of human cervical cancer cells[7], medullary thyroid malignancy cells[6,8] and human small intestinal neuroendocrine tumour cells[6]. In fact, it is regularly the situation that phytochemical compound from restorative plants help to counter multiplication of malignant growth by inciting apoptosis[9]. The extractions of phytochemical substances that contain anticancer properties rely upon numerous factors, for example, types of solvents and method used. Alluding to the previous research directed by Nguyen-Pouplin[7], the use of cyclohexane as extraction solvent on *C. vespertilionis* showed cytotoxicity effect on the treated cancer cell. However, in a similar report, no cytotoxic activities of *C. vespertilionis* was seen when difference solvent extraction, for example, methylene chloride, methanol and crude ethanol extract solvent extractions were utilized. In spite of the capability of *C. vespertilionis* to induce cytotoxicity towards cancer cell line, little data exists on the cytotoxic effects of the extracts of this plant. To close the gap, the present research has investigated the cytotoxic activities of *C. vespertilionis* extracts and detected the phytochemical compound present in the extract utilizing gas chromatography-mass spectrometry (GC-MS) analysis.

MATERIAL AND METHODS

Materials

Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS) and Penicillin-Streptomycin were purchased from Gibco®, USA. Dimethyl sulfoxide (DMSO) was obtained from Sigma Aldrich (St. Louis, USA). Methanol, hexane, dichloromethane, chloroform and n-butanol were obtained from Fisher Scientific, UK

Plant material

C. vespertilionis plants were gathered from Guar Perahu Herbal Valley, Penang, Malaysia (5.426404 °N, 100.478224 °E). *C. vespertilionis* was recognized utilizing universal DNA barcode primers as portrayed by Ismail[10]. The purified PCR products were packed and sent for sequencing purposes. All DNA sequences were distinguished utilizing the Basic Local Alignment Search Tool (BLAST) database from the National Centre for Biotechnology Information (NCBI). In addition, the plant was likewise recognized by Dr. Rahmad Zakaria from the herbarium of the School of Biological Sciences, Universiti Sains Malaysia and the voucher number 11777 was obtained (supplementary data). The fresh leaves were washed and weighed with refined water. Crisp leaves were exposed to air drying for 5 days in the research facility at room temperature of 25–30 ºC. The dried leaves were ground into coarse powder to expand the extraction yield.

Preparation of extracts

The 42.86 g powdered sample of *C. vespertilionis* was extracted with 80% methanol utilizing the maceration strategy. The sample was then filtered and extracted again with a similar solvent to guarantee total extraction for 3 days. Rotary evaporator and freeze-dried instrument then was used to evaporate any solvent left in the extract. Next, the dried crude extract was re-suspended with refined water and after that exposed to fractionation extractions utilizing various solvents of increasing polarity, utilizing hexane, dichloromethane, chloroform and n-butanol solvents. The fractionation was repeated multiple times to ensure maximum extraction yield from each partitioned extract can be obtained, and the separation between two immiscible phases were separated based on the density of solvent used. The fractions or partitions then were dried utilizing a freeze dryer (Genevac Ltd. UK) to yield hexane, dichloromethane (DCM), chloroform, n-butanol and aqueous extracts. The fraction extracts were stored in -20°C.

Cell cytotoxicity assay

Sulforhodamine B (SRB) assay was used to determine cytotoxic activity of each extracts[11,13]. Cervical cancer cells (HeLa) were cultured in DMEM medium and incubation was conducted fewer than 5% CO_2 at 37°C for 48-72 h to reach 80% confluence. After reaching 80% confluency, the cells were seeded in 96 well plates and incubated for another 24 h for fully attachment. The density of cell was kept at $3x10^4$ for each well in 96 well plates. The extracts were prepared freshly before exposing the extract to the HeLa cells. All the insoluble extracts were prepared with less than 0.1% DMSO to prevent toxicity to the cell. After 24 h attachment of the cells to the well of 96 well plates, the cells were treated with each extracts (crude extract and fraction extracts such as hexane, dichloromethane, chloroform, and aqueous residue). Five concentration (difference concentrations for each extracts based on pre-trial results) of each extract with 4 replicate was set up. Positive and negative control was also conducted to ensure validity of results. Paclitaxel was used as positive control while negative control used media alone or together with 0.1% DMSO.

Cytotoxicity activities of every extract were recorded for each time point (24, 48 and 72 hours). Addition of 50 µL trichloroacetic acid (TCA) solution was conducted on each well plates containing HeLa cell line and incubation was done in ambient temperature for about 30 minutes to fix the cells for each time point (24, 48, and 72 hour). Following 30 minutes, the plates were washed with water and after that dried at room temperature. Cells were stained with 100 µl SRB reagent and incubated for 30 minutes at room temperature. After incubation, the plates were washed with 1% acetic acid and after that dried. At that point, the cells were washed with washing buffer, 10 mMTr is buffer and after that shaken the plates for 5 minutes. The plates were examined in absorbance readerat 540 nm wavelengths. The half maximal inhibitory concentration (IC_{50}) of the plant extraction was dictated by calculating the cell viability utilizing the accompanying formula: Cell Viability (%) = [Average Absorbance (Sample-Blank)] / [Average Absorbance (Negative Control-Blank)] x 100.

Morphology Changes of HeLa Cell

Olympus inverted microscope (Eclipse TE 300; Olympus, Tokyo, Japan) was used to observe and recorded the cytotoxic effects of the selected *C. vespertilionis* extract (the extract that created lowest IC_{50} value) on cell morphology of treated HeLa cells. Extract of *C. Vespertilionis* was used to treat HeLa cell line ($3x10^4$ cells/mL) and incubation period was done for 24, 48 and 72 hours in 96-well plates. After every time point, observation towards cell morphology was conducted and the images were recorded (Olympus 12-bit CCD camera, Olympus).

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of phytochemical compound of selected extracts of *C. vespertillionis* was conducted using Agilent Technologies 6890 N Network GC system for gas chromatography. The analysis was started by dissolving 5 g of the extract in 1 mL methanol and then filtered it using 0.45 µm Whatman nylon syringe filter. Prepared extracts (1µL) in the vial was auto injected in a split less mode. The initial temperature was

set at 70ºC and hold for 2 minutes. The temperature then increased to 160ºC with increasing rate of 10ºC/min and holding time about 5 minutes. Finally, the temperature was increased to 270ºC at 20ºC/min and maintained for 8 minutes. The percentage of relative abundance area of each identified compounds from National Institute of Standards and Technology (NIST) library data were calculated.

Statistical analysis

All analyses were conducted in four replicate and these values were presented as average values along with their standard deviation. Graph Pad Prism version 7.0 was used to plotgraphs (cell viability (%) vs log concentration) in order to determine the IC_{50} of the extract's groups and positive control groups. Kruskall Wallis (non-parametric) test was used to compare the viability of the cell (%) between the treatment groups with untreated group (negative control). All variables were analyzed through SPSS and Graph Pad Prism 7 with $p < 0.05$ considered as significant.

RESULTS

Extraction of *C. vespertilionis*

The percentage yield of crude extracts from 80% methanol was 11.51%. The fraction extracts were varied from 4.65% to 54.31% according to the following ascending order, chloroform (4.65%) <hexane (13.38%) <dichloromethane (32.48%) <*n*-butanol (35.03%) < aqueous residue (54.31%) (Table 1).

Table 1: Percentage yield of *C. vespertilionis* crude and partition extracts

Extracts	Dry weight (g)	Extraction yield (%) [a]
Crude (80% Methanol)	4.933	11.51
Partitioned extracts	**Dry weight (g)**	**Extraction yield (%) [b]**
Hexane	0.660	13.38
Dichloromethane	1.602	32.48
Chloroform	0.229	4.65
n-butanol	1.728	35.03
Aqueous residue	2.679	54.31

[a] Extraction yield is expressed as 100 x [dry extract (g)/dry leaves (g)].
[b] Extraction yield is expressed as 100 x [partitioned extract (g)/ crude extract (g)].

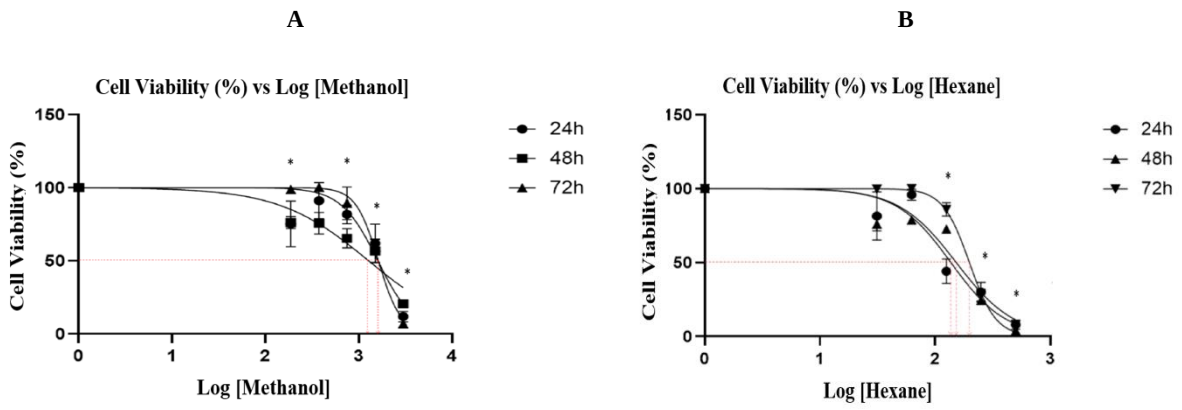
***C. vespertilionis* extracts showed significant cytotoxicity effect against the HeLa**

The percentage of cell viability was observed at 24, 48, 72 hours using SRB assay. Table 2 showed half inhibition concentration (IC_{50}) for crude methanol and partition extracts on human cervical cancer cells (HeLa). The determination of IC_{50} was conducted by plotting a graph of percentage of cell viability versus different concentration in logarithm (Figure 2). The IC_{50} of the *C. vespertilionis* extract on the cervical cancer cell (HeLa) that produced high toxicity was dichloromethane (DCM) extract by 63.68 µg/mL ± 3.46, 55.42 µg/mL ± 3.73 and 53.04 ± 3.29 µg/mL at 24, 48 and 72 hours respectively.

Table 2: Half inhibitory concentration (IC_{50}) of *C. vespertilionis* extracts for each time point

	Time point (Hours)		
Extract	**24**	**48**	**72**
Methanol	1603 µg/mL ± 10.25[a]	1275 µg/mL ± 5.62	1623 µg/mL ± 4.10
Hexane	136.8 µg/mL ± 7.62	152.2 µg/mL ± 2.33	198.0 µg/mL ± 2.12
Dichloromethane	63.68 µg/mL ± 3.46	55.42 µg/mL ± 3.73	53.04 µg/mL ± 3.29
Chloroform	192.4 µg/mL ± 3.30	813.8 µg/mL ± 6.27	828.9 µg/mL ± 4.14
n-Butanol	- µg/mL	- µg/mL	- µg/mL
Aqueous	- µg/mL	- µg/mL	- µg/mL
Paclitaxel	0.02707 µg/mL ± 4.76	0.02665 µg/mL ± 7.51	0.02641 µg/mL ± 5.77

[a]Data are presented as mean ± SD



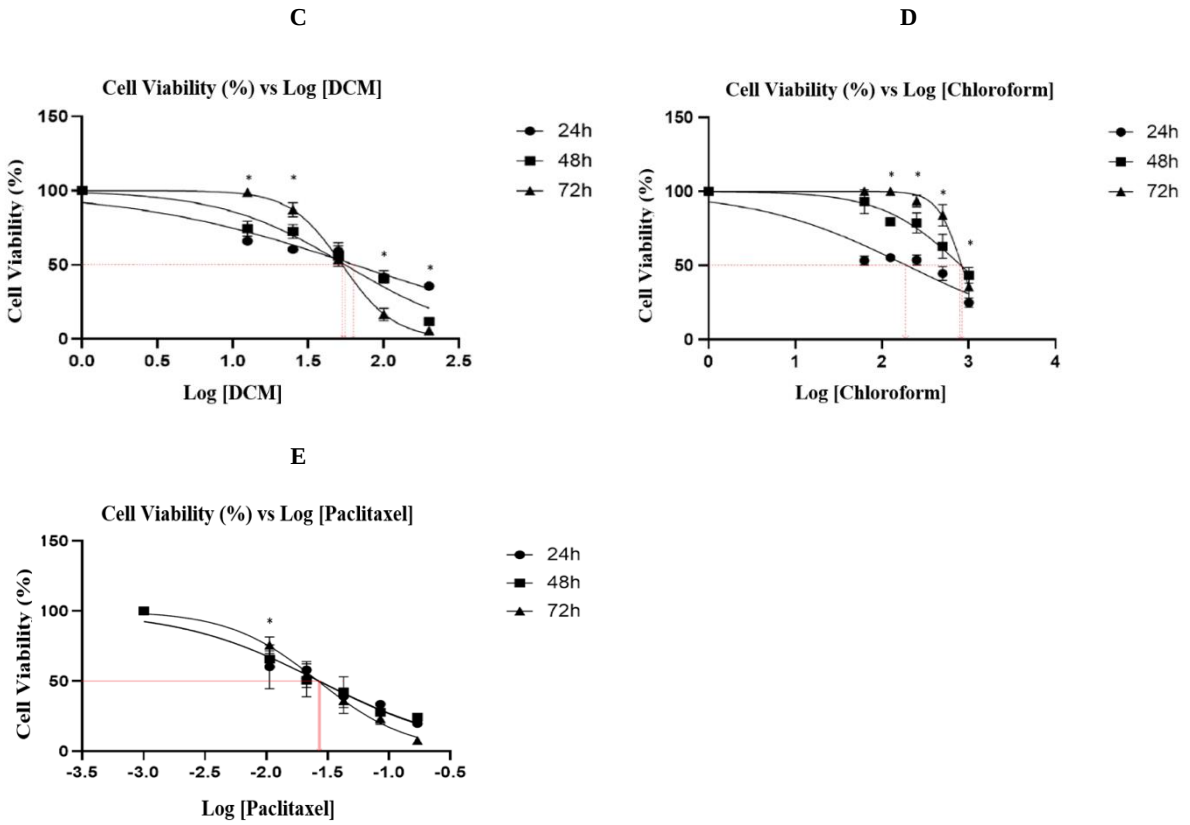


Figure 2: (A) Dose response relationship between cell viability (%) with log concentration of crude methanol extract on the human cervical cancer cell (HeLa) for every time point. (B) Dose response relationship between cell viability (%) with log concentration of hexane extract on the human cervical cancer cell (HeLa) for every time point. (C) Dose response relationship between cell viability (%) with log concentration of dichloromethane (DCM) extract on the human cervical cancer cell (HeLa) for every time point. (D) Dose response relationship between cell viability (%) with log concentration of chloroform extract on the human cervical cancer cell (HeLa) for every time point. (E) Dose response relationship between cell viability (%) with log concentration of paclitaxel (positive control) on the human cervical cancer cell (HeLa) for every time point

Data are presented as mean ± SD, * $p < 0.05$

Morphological Characteristics of the Cell

Human cervical cancer cell (HeLa) was immortalised and adherent cell line that owned spindle-shaped. The effects of DCM extract on morphological changes and characteristics of HeLa cells were provided in this section. Inverted microscope was used to capture the image (Figure 3 and Figure 4).

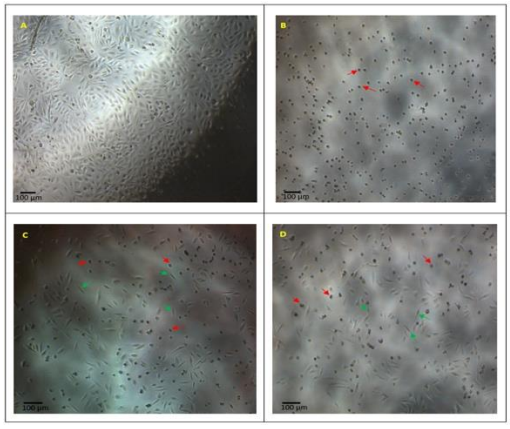


Figure 3: Morphological characteristic of HeLa under magnification of 4x. The red arrows indicated possible apoptotic bodies of the cancer cell. The green arrow showed normal spindle shape of HeLa cell line. (A) Morphological characteristic of HeLa before treatment. (B) Morphological characteristic of HeLa after treatment with 200 µg/mL dichloromethane (DCM) *C. Vespertilionis* extract. (C) Morphological characteristic of HeLa after treatment with 100 µg/mL DCM *C. Vespertilionis* extract. (D) Morphological characteristic of HeLa after treatment with 50 µg/mL DCM *C. vespertilionis* extract

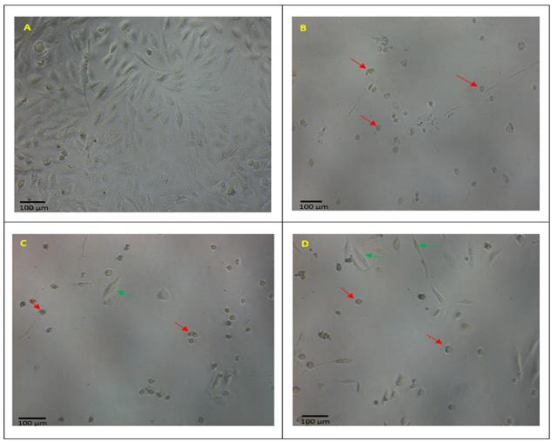


Figure 4: Morphological characteristic of HeLa under magnification of 10x. The red arrows indicated possible apoptotic bodies of the cancer cell. The green arrow showed normal spindle shape of HeLa cell line. (A) Morphological characteristic of HeLa before treatment. (B) Morphological characteristic of HeLa after treatment with 200 µg/mL dichloromethane (DCM) *C. vespertilionis* extract. (C) Morphological characteristic of HeLa after treatment with 100 µg/mL DCM *C. vespertilionis* extract. (D) Morphological characteristic of HeLa after treatment with 50 µg/mL DCM *C. vespertilionis* extract

Gas Chromatography-Mass Spectrometry

GC-MS analysis was conducted on the *C. vespertilionis* DCM extract to screen the possible phytochemicals present in the DCM extract. The major bioactive compounds present in the extract obtained from *C. Vespertilionis* leaves were separated using gas chromatography (GC) and identified through mass spectrometry (MS). Figure 5 showed the peaks of the *C. vespertilionis* extract analysed by the GC-MS and the result was tabulated in Table 3. Meanwhile possible activity of major phytochemical compound from DCM extract were tabulated in Table 4

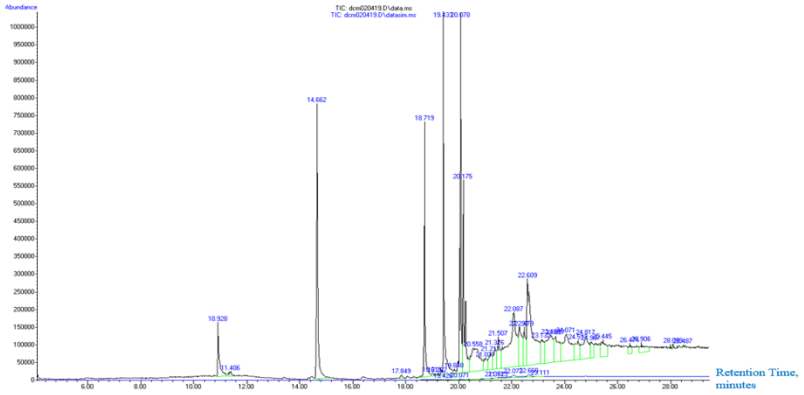


Figure 5: GC-MS profile of dichloromethane (DCM) leaf extract of *C. vespertilionis*

Table 3: Phytochemical components identified in DCM leaves extract of *C. vespertilionis* using GC-MS

Peak	Compound	RT	Molecular Formula	Molecular Weight (g/mol)	Area (%)
1	n-Dodecyl acetate	10.928	$C_{14}H_{28}O_2$	228.209	2.0448
2	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	11.406	$C_{13}H_{18}O_2$	206.131	0.3625
3	2-Propenoic acid, pentadecyl ester	14.662	$C_{18}H_{34}O_2$	282.256	8.7497
4	2-Pentadecanone, 6,10,14-trimethyl-	17.849	$C_{18}H_{36}O$	268.277	0.2026
5	Hexadecanoic acid, methyl ester	18.719	$C_{17}H_{34}O_2$	270.256	4.8738
6	1-(4-Methoxy-phenyl)-5,5-dioxo-hexahydro-5.lambda.(6)-thieno[3,4-b]pyrrol-2-one	19.019	$C_{13}H_{15}NO_4S$	281.072	0.1648
7	p-Anisaldehyde 4-[1-adamantyl]-3-thiosemicarbazone	19.202	$C_9H_{11}N_3OS$	343.172	0.0795
8	Cyclodecane	19.433	$C_{12}H_{24}$	168.188	7.0353
9	Ethyl (1-adamantylamino) carbothioylcarbamate	19.848	$C_{14}H_{22}N_2O_2S$	282.14	0.2421
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	20.078	$C_{20}H_{34}O_2$	292.24	7.8162
11	Phytol	20.175	$C_{20}H_{40}O$	296.308	6.9703
12	Pyrazole-5-carboxamide, N-(1,3-benzodioxol-5-ylmethyl)-1-ethyl-	20.558	$C_{14}H_{15}N_3O_3$	273.111	4.924
13	2-Ethylacridine	21.021	$C_{15}H_{13}N$	207.105	0.9425
14	1-Dimethyl(phenyl)silyloxypentane	21.211	$C_{13}H_{22}OSi$	222.144	1.3495

15	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	21.376	$C_{21}H_{25}NO_4$	355.178	1.354
16	5-(p-Aminophenyl)-4-(O-tolyl)-2-thiazolamine	21.507	$C_{16}H_{15}N_3S$	281.099	1.9733
17	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	22.087	$C_{14}H_{44}O_6Si_7$	504.152	9.8818
18	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	22.297	$C_{21}H_{25}NO_4$	355.178	3.2151
19	Benzamide, N-(2,4-dinitrophenyl)-3,5-dinitro-	22.479	$C_{13}H_9N_3O_5$	377.024	2.0562
20	Anthracene, 1-methyl-	22.609	$C_{15}H_{12}$	192.094	10.5844
21	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	23.132	$C_{21}H_{25}NO_4$	355.178	1.9181
22	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	23.488	$C_{14}H_{44}O_6Si_7$	504.152	4.6507
23	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	23.657	$C_{21}H_{25}NO_4$	355.178	3.0863
24	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	24.071	$C_{21}H_{25}NO_4$	355.178	5.2966
25	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	24.513	$C_{21}H_{25}NO_4$	355.178	1.8205
26	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	24.817	$C_{14}H_{44}O_6Si_7$	504.152	3.4581
27	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	24.997	$C_{14}H_{44}O_6Si_7$	504.152	1.1178
28	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	25.445	$C_{17}H_{14}O_4$	282.089	1.9053
29	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	26.474	$C_{14}H_{44}O_6Si_7$	504.152	0.5425
30	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	26.906	$C_{21}H_{25}NO_4$	355.178	1.1891
31	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	28.099	$C_{21}H_{25}NO_4$	355.178	0.1444
32	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	28.487	$C_{21}H_{25}NO_4$	355.178	0.0482

Table 4: Activity of major phytochemical compounds identified in *C. vespertilionis* leaves of DCM extract by GC-MS

RT (Minutes)	Compound name	Nature of Compound	Activity
14.662	2-Propenoic acid, pentadecyl ester	Acrylic acid ester	Antimicrobial, Antioxidant[32]
18.719	Hexadecanoic acid, methyl ester	Palmitic acid ester	Antioxidant[32]
19.433	Cyclododecane	Organic compound	Antimicrobial, Antitumour[33]
20.078	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Linolenic acid	Anti-inflammatory, antihistaminic[32]
20.175	Phytol	Diterpene	Antimicrobial, anticancer, anti-inflammatory[32]

DISCUSSION

Cytotoxicity activity of *C. vespertilionis* crude methanol and partitioned extracts were explored on HeLa cell lines by SRB assay[11,12,13]. In term of half inhibitory concentration (IC_{50}), dichloromethane (DCM) extract of *C. vespertilionis* demonstrated the least IC_{50} value. Half inhibitory concentration alluded to the capacity of the compound to hinder half of the number of population of cell line[14]; the lower the IC_{50}, the higher the level of cytotoxic effect of the extract. The outcomes demonstrated that the DCM extract of the plant has promising half inhibitory activities on HeLa cell lines by 63.68, 55.42 and 53.04 µg/mL at 24, 48 and 72 hours. Numerous research had shown that DCM sub-fraction extract displayed high cytotoxicity to the cancer cell line compared with other sub-fraction extract[17,15,16]. In the interim, the other extracts, for example, hexane, chloroform and crude methanol extracts had delivered higher IC_{50} than DCM extract for each time point (Table 2). n-butanol and aqueous extract of *C. vespertilionis* be that as it may, failed to demonstrate any IC_{50} value even after 4000 µg/mL of the extracts have been exposed on HeLa cell until 72 hours.

In the other study, the IC_{50} value of cyclohexane extract of *C. vespertilionis* leaves on HeLa cell was recorded as 9.9 µl/mL at 72 hours[7]. The discoveries from the present research however demonstrated that the most minimal IC_{50} obtained was 53.04 µg/mL by DCM extract. The IC_{50} value finding in this research was multiple times higher contrasted with the past discovery. The components that may contribute to this differences possibly due to differences of plants collection places, strategy for extraction and so forth[18,19]. The past examination demonstrated that distinction of IC_{50} was displayed by different plant separate when different extraction solvent was directed for each plant[20]. In term of promising agents for anticancer medication advancement, U.S. National Cancer Institute (USNCI) has expressed that lone extracts with IC_{50} estimations of under 30 µg/mL against exploratory tumour cell lines are considered as promising agents for anticancer medication development[21,22].

The information from this study likewise demonstrated that half inhibitory concentration was time dependent. In this research, IC_{50} of positive control (paclitaxel) demonstrated the diminishing pattern when the HeLa was presented to the chemo drug for each time point (24, 48 and 72 hours). The comparative pattern was appeared by DCM extract of *C. vespertilionis* leaves. The other extracts anyway did not demonstrate the comparable patterns. As indicated by the previous study, one of the attributes or characteristics to create anticancer drug depended on the concentration and time in which the viability of cancer cell line must be decreased as the concentration and time expanded[23,24].

Gas chromatography-mass spectrometry investigation was directed to screen for the potential phytochemicals which were presented in the DCM extract of *C. vespertilionis* which is utilized

in this study. This prominent phytochemical compound may be the explanation behind the cytotoxic activities of the extracts onto the HeLa cell lines. The major phytochemical compound that were screened in the *C. vespertilionis* leaves extract utilizing GC-MS analysis were 2-propenoic acid, hexadecanoic acid, cyclododecane, 9,12,15-octadecatrienoic acid and phytol.

The dispersion and distribution of these phytochemical compounds were represented by the principle of "like dissolve like". The principle of "like dissolve like" was alluding to the abilities of solvents to disperse and distribute the compound dependent on their polarity[25]. For instance, polar compound will be circulated in polar solvent and non-polar compound will be disseminated in less or non-polar solvent. Be that as it may, not all the compound will pursue this principle. Many factors can add to this issue; for instance, the nature of the compound itself, explicitness of technique utilized and the issue of detector. Some compound particularly polar compound was not reasonable to be distinguished by specific instruments, for example, GC-MS. GC-MS relied upon the temperature to actuate the analyte to be volatilized. Derivative agents, for example, trimethylsilyl (TMS) derivatise can be utilized to quantitate less volatile compound for GC examination[26].

Most of the phytochemical compound presented in the extract contained anti-inflammatory and anti-oxidant activities. A few studies have demonstrated that 2-propenoic acid, hexadecanoic acid, and 9,12,15-octadecatrienoic acid conceivably contain anti-inflammatory activities[27-29]. According to the past research, cancer development can be prevented by reducing the secretion of inflammatory compound utilizing anti-inflammatory compound while formation of reactive oxygen species (ROS) can be prevented through anti-oxidant activities[30,31]. This study anyway was only an early screening and more examinations expected to affirm the real exercises of the phytochemical compound. Further investigation of these phytoconstituents utilizing progressive and sensitive detector, for example, HPLC, LC-MS and NMR may demonstrate their therapeutic significance later on.

CONCLUSION

In conclusion, highest cytotoxicity activity indicated by the lowest IC_{50} was shown by DCM extract of *C. vespertilionis* compared to others extracts. Plus, the presence of apoptotic bodies in the treated HeLa cell line and presence of various phytochemical compounds that owned specific activities such as anti-inflammatory, antioxidant and anticancer activities may propose the cytotoxicity of the extract.

ACKNOWLEDGEMENT

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REFERENCES

1. List T.P. Version 1.1 Published on the Internet; http://www.theplantlist.org/ (accessed 1st January); 2013.
2. Nurul S.A.S., Hazilawati H., Mohd R.S., Mohd F.H.R., Noordin M.M. and Norhaizan M.E. Subacute oral toxicity assesment of ethanol extract of *Mariposa christia vespertilionis* leaves in male Sprague Dawley rats. Toxicological research 2018; 34(2): 85.
3. Bunawan H., Bunawan S.N. and Baharum S.N. The Red butterfly wing (*Christia vespertilionis*): A promising cancer cure in Malaysia. Int J Pharm Pharm Sci 2015; 7(8): 1.
4. Upadhyay H.C., Sisodia B.S., Cheema H.S., Agrawal J., Pal A., Darokar M.P. and Srivastava S.K. Novel antiplasmodial agents from *Christia vespertilionis*. Natural Product Communications 2013; 8(11): 1591-1594.
5. Dash G.K. An appraisal of *Christia vespertilionis* (LF) bakh. F.: A promising medicinal plant. Int. J. of Pharmacognosie and Phytochem Research 2016; 8(6): 1037-1039.
6. Hofer D., Schwach G., Ghaffari Tabrizi-Wizsy, N., Sadjak A., Sturm S., Stuppner H. and Pfragner R. *Christia vespertilionis* plant extracts as novel antiproliferative agent against human neuroendocrine tumor cells. Oncology reports, 2013; 29(6): 2219-2226.
7. Nguyen-Pouplin J., Tran H., Tran H., Phan T.A., Dolecek C., Farrar J., Tran T.H., Caron P., Bodo B and Grellier P. Antimalarial and cytotoxic activities of ethno pharmacologically selected medicinal plants from South Vietnam. Journal of Ethnopharmacology 2007; 109(3): 417-427.
8. Strasser M., Phytochemische Untersuchung von Christia vespertilionis; 2010: na.
9. Wang H., Oo Khor T., Shu L., Su Z.Y., Fuentes F., Lee J.H. and Tony Kong A.N. Plants vs. cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 2012; 12(10): 1281-1305.
10. Ismail N.Z., Arsad H., Samian M.R., Hamdan M.R. and Othman A.S. Assessment of three plastid DNA barcode markers for identification of *Clinacanthus nutans* (Acanthaceae). 3 Biotech 2018; 8(1): 62.
11. Bhagat J., Lobo R., Kumar N., Mathew J.E. and Pai A. Cytotoxic potential of *Anisochilus carnosus* (Lf) wall and estimation of luteolin content by HPLC. BMC complementary and alternative medicine 2014; 14(1): 421.
12. Orellana E.A. and Kasinski A.L. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio-protocol 2016; 6(21).
13. Lopez D., Cherigo L., de Sedas A., Spadafora C. and Martinez-Luis, S. Evaluation of antiparasitic, anticancer, antimicrobial and hypoglycemic properties of organic extracts from Panamanian mangrove plants. Asian Pacific Journal of Tropical Medicine 2018; 11(1): 32.
14. Smith E., Palethorpe H., Tomita Y., Pei J., Townsend A., Price T., Young J., Yool A. and Hardingham J. The purified extract from the medicinal plant *Bacopa monnieri*, bacopaside II, inhibits growth of colon cancer cells *in vitro* by inducing cell cycle arrest and apoptosis. Cells 2018; 7(7): 81.
15. Pasquet V., Morisset P., Ihammouine S., Chepied A., Aumailley L., Berard J.B., Serive B., Kaas R., Lanneluc I., Thiery V. and Lafferriere M. Antiproliferative activity of violaxanthin isolated from bioguided fractionation of *Dunaliella tertiolecta* extracts. Marine drugs 2011; 9(5): 819-831.
16. Thani W., Vallisuta O., Siripong P. and Ruangwises N. Anti-proliferative and antioxidative activities of Thai noni/Yor (*Morinda citrifolia* Linn.) leaf extract. Southeast Asian J Trop Med Public Health 2010; 41(2): 482-9.
17. Yaacob N.S., Hamzah N., Kamal N.N.N.M., Abidin S.A.Z., Lai C.S., Navaratnam V and Norazmi M.N. Anticancer activity of a sub-fraction of dichloromethane extract of *Strobilanthes crispus* on human breast and prostate cancer cells *in vitro*. BMC complementary and alternative medicine 2010; 10(1): 42.
18. Khoo L.W., Audrey Kow S., Lee M.T., Tan C.P., Shaari K., Tham C.L. and Abas F. A Comprehensive Review on Phytochemistry and Pharmacological Activities of

Clinacanthus nutans (Burm. f.) Lindau. Evidence-Based Complementary and Alternative Medicine; 2018.
19. Mendez-Flores, A., Hérnandez-Almanza, A., Sáenz-Galindo, A., Morlett-Chávez, J., Aguilar, C.N. and Ascacio-Valdés, J. Ultrasound-assisted extraction of antioxidant polyphenolic compounds from *Nephelium lappaceum* L.(Mexican variety) husk. Asian Pacific Journal of Tropical Medicine 2018; 11(12): 676.
20. Talib W and Mahasneh A. Antiproliferative activity of plant extracts used against cancer in traditional medicine. Scientia Pharmaceutica 2010; 78(1): 33-46.
21. Suffness M. Assays related to cancer drug discovery. Methods in plant biochemistry: assays for bioactivity 1990; 6: 71-133.
22. de SS Quintans, J, Soares B.M., Ferraz R.P., Oliveira A.C., da Silva T.B., Menezes L.R., Sampaio M.F., Prata A.P.D.N., Moraes M.O., Pessoa C. and Antoniolli, A.R. Chemical constituents and anticancer effects of the essential oil from leaves of *Xylopia laevigata*. Planta medica, 2013; 29(02): 123-130.
23. Huschtscha L.I., Bartier W.A., Ross, C.E. and Tattersall M.H.N. Characteristics of cancer cell death after exposure to cytotoxic drugs *in vitro*. British Journal of Cancer 1996; 73(1): 54.
24. Solowey E., Lichtenstein, M., Sallon, S., Paavilainen, H., Solowey, E. and Lorberboum Galski H. Evaluating medicinal plants for anticancer activity. The Scientific World Journal; 2014.
25. Kim H.K. and Verpoorte R. Sample preparation for plant metabolomics. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 2010; 21(1): 4-13.
26. Popova M.P., Graikou K, Chinou I and Bankova V.S. GC-MS profiling of diterpene compounds in Mediterranean propolis from Greece. Journal of Agricultural and Food Chemistry 2010; 58(5): 3167-3176.
27. Kumar P.P., Kumaravel S. and Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. African Journal of Biochemistry Research 2010; 4(7): 191-195.
28. Aparna V, Dileep KV, Mandal PK., Karthe P., Sadasivan C. and Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology and Drug Design 2012; 80(3): 434-439.
29. Hai Dang, N., Choo, Y.Y., Tien Dat, N., Hoai Nam, N., Van Minh, C. and Lee, J.H. 7-Methoxy-(9H-β-Carbolin-1-il)-(E)-1-Propenoic Acid, a β-Carboline Alkaloid From *Eurycoma longifolia*, Exhibits Anti-Inflammatory Effects by Activating the Nrf2/Heme Oxygenase-1 Pathway. Journal of Cellular Biochemistry 2016; 117(3): 659-670.
30. Calixto JB., Campos MM., Otuki M.F and Santos AR. Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta medica 2004; 70(02): 93-103.
31. Abdul WM, Hajrah N.H., Sabir JS., Al Garni S.M., Sabir M.J., Kabli S.A., Saini K.S. and Bora RS. Therapeutic role of *Ricinus communis* L. and its bioactive compounds in disease prevention and treatment. Asian Pacific Journal of Tropical Medicine 2018; 11(3): 177.
32. Mujeeb F., Bajpai P and Pathak N. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. BioMed Research International; 2014.
33. Paramanantham M. and Murugesan A. GC-MS analysis of *Holarrhena antidysentrica* Wall flower. International Journal of Science Engineering and Technology Research 2014; 3(3): 631-39.

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