ORIGINAL ARTICLE

Phytochemicals Screening and Anti-proliferative Activities of *Anacardium occidentale* Shoot Extract in Breast Cancer Cells

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ABSTRACT

Introduction: *Anacardium occidentale* or cashew are popular traditional food and have raised research interest for complementary cancer treatment. Cancer has become leading cause of death and treatment involved severe side effects. In present study, we aim to study the anti-proliferative effects of cashew shoots in breast cancer (MDA-MB-231), colorectal cancer (HT-29) and liver cancer (HepG2) cell lines. **Methods:** Cell lines were treated with 70% ethanolic cashew extract for cytotoxicity test with MTT assay. AO/PI dual fluorescent assay and RNase/PI staining were used to determine apoptosis induction effects. Phytochemicals screening was carried out by using gas chromatography mass spectrometry (GCMS) and liquid chromatography mass spectrometry (LCMS). **Results:** The cytotoxicity assay of cashew shoot extract demonstrated IC_{50} of 81.1 ± 0.11 µg/ml for MDA-MB-231, 307.5 ± 2.31 µg/ml for HT-29 and 272.6 ± 1.91 µg/ml for HepG2 cell lines. The apoptotic bodies include chromatin condensation, cell blebbing and nuclear fragmentation and apoptosis induction were shown by AO/PI staining. There was significant increase of cell count in sub-G0 phase in MDA-MB-231 cell lines treated with cashew shoot extract. It was demonstrated that cashew shoot extract contained 38 compounds from GCMS such as sitosterol, tannin, pyrogallol, phenol and 20 compounds from LCMS such as citric acid, gallic acid, myricetin and hinokiflavone that may give rise to its anti-cancer effect. **Conclusion:** Cashew shoot extract demonstrated potential anti-cancer properties thus further study is required to investigate its mechanism as anti-cancer agent.
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Keywords: *Anacardium occidentale*, Shoot, Anticancer, Apoptosis, Phytochemicals

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INTRODUCTION

Cancer is a public health concern and has become one of the top causes of mortality in the world. According to the epidemiology trend for the leading cause of mortality from 2016 to 2060, expected by WHO, the cancer mortality rate will increase 2.08-fold by 2060. Until then, malignancies will become the most common cause of mortality worldwide by as early as 2030 (1). Cancer is defined as the quick formation of a cell anomaly that grows into a tumour (2). Breast, colorectal and liver cancer are the highest cancer incidence worldwide. These cancers are the top five commonest cancer worldwide and the global incidence were rising (3). According to Malaysia National Cancer Registry Report 2012 – 2016, breast cancer has the highest cancer incidence in Malaysia and has remained its crown since 2007. In addition, it is also the most common cancer among female and have seen an increasing trend among young female. Meanwhile, colorectal cancer is the commonest cancer among Malaysian males and the second commonest cancer among Malaysian females (4). Furthermore, liver cancer was reported as the top 5 most frequent cancer in both males and females, with 3rd in overall cancer mortality (4). The common cancer treatment includes chemotherapy, surgery and radiotherapy that typically have severe side effect and affect the quality of life of patients (5). Lately, research has focused on drug discovery and development for cancer involving stimulation of programmed cell death (6). Traditional medicinal plants can synthesise a great mixture of chemical compounds can be used as alternative source for western medicine and therapeutic remedies as they are inexpensive and natural (7). Therapeutics approach derived from plant compounds or phytochemicals as an alternative for cancer have been administered extensively. It is suggested that phytochemical compounds isolated from medicinal plants contains key to leads towards anti-cancer

medications. For example, the polyphenols from fruits and vegetables exhibit chemopreventive effects that are reported to exhibit anticancer activity (8).

Anarcadium occidentale or cashew plant is under the family of *Anarcadiaceae*. It is mainly distributed in tropical, subtropical and temperate areas and is the most common cultivated and used species among the Anacardiaceae family (9). Different part of the cashew plant is comprised of different nutrients, for example, cashew leaf contains quercetin as dominant flavonoid and chlorogenic acid as the dominant phenolic acid, cashew nuts are rich in unsaturated fatty acids such as linolenic and oleic acid, while the cashew shoot contains flavonoids (10). It is reported that the leaves of cashew possess many medicinal properties that includes anti diabetic, antimicrobial, anti-fungi, anti-inflammatory (11). The young leaves and shoots of cashew plants were consumed in Malaysian community as a side dish or salad that also known as ulam. These vegetables were eaten either raw or cooked as accompaniment with main meal. It is an important component of traditional diet. Traditionally, cashew shoots were suggested to have medicinal properties and the knowledge was passed down through generations despite no or little scientific evidence to support the claim (12). While the prevalence of cancer is high among the population, the current treatment and therapeutic intervention are high in cost and cause severe side effect that affect the quality of life of cancer patients. One of the efforts for effective treatment that is free from side effects involved natural bioactive phytochemicals from plants and herbs that exhibit anti-cancer properties that can be introduced as part of daily dietary intake (13). Although cashew has been studied extensively, most of the studies focus on other parts of the plant, for instance, nuts, fruits, leaves and bark, for antibacterial, anti-fungal, antioxidant and anti-inflammatory properties (10), therefore further studies on the anticancer and phytonutrients in cashew shoots can contribute to overall benefits of cashew were investigated in this study.

MATERIALS AND METHODS

Sample Collection and Extraction

Anarcadium occidentale L. shoots were obtained fresh from Taman Herba, Universiti Putra Malaysia (UPM). It was verified with the voucher number SK 3216/17 for cashew shoot. The samples were handled on the same day. The cashew shoot samples were separated and washed. Prior to freeze drying using freeze dryer, the samples were stored in -20 °C in freezer overnight then proceed for freeze frying at 0.5 psi in a freeze dryer (Heto Power Dry PL3000, Thermo Fisher Scientific, Japan). The samples were grounded into powder form and extracted with 70 % ethanol. Two grams (2g) of freeze-dried cashew shoot powder was mixed with 100 ml of 70% ethanol. The mixture is undergoing centrifugation at 1536 x g for 5 minutes. Rotary evaporator was used to remove the supernatant and the extract were dissolved in distilled water (1mg/ml) supplemented with 5% dimethyl sulfoxide (DMSO) for cell culture treatment (14).

Cell culture

Breast cancer cell lines as MDA-MB-231, colorectal cancer cell lines as HT-29, liver cancer cell lines as HepG2 and normal mouse fibroblast cells as BALB/c 3T3 were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). They were cultured in RPMI supplemented with 10 % (v/v) fetal bovine serum (FBS) and antibiotics (100 µg/ml streptomycin and 100 IU/ml penicillin). Cells were grown in cell culture flask at 37 °C in a humidified atmosphere with 5 % CO2 atmosphere. The subculture was performed when the cells reached 70-80% confluency to avoid cell death. The passage number of the cells that were used in this study was below 20.

MTT assay

MTT refers to (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). MTT assay was used to examine the cells proliferation. All the cell lines were seeded in a 96-well microtiter plate and treated with several concentrations of cashew shoot extract range from 0 – 1000 µg/ml (supplemented with 0.5% of DMSO/well) with for 72 hours. The old media was discarded after 72 hours of incubation and MTT solution was added to each well and further incubate for 4 hours in the dark. Microplate reader was used to read the absorbance at 570 nm wavelength. The 50 % growth inhibition concentration (IC_{50}) values were determined in which graph was being plotted with the percentage the viable cells against their respective concentrations of extracts. The endpoint criteria for anti-proliferative activity for the crude extracts with $IC_{50} < 100$ µg/ml as promising anti-cancer agent was selected for further analysis (15,16).

Acridine orange/Propidium iodide (AO/PI) assay

AO/PI assay were among the most common used fluorescent dyes for analyzing cell culture viability for living, apoptotic and late apoptosis states. Quantification of apoptosis was performed by using AO/PI assay as previously demonstrated with adjustments (17). The chosen cell lines were seeded at density 1 x 10^6 cells/ml in 6-well plates and were treated with IC_{50} concentrations of cashew shoot extract from MTT assay. It was then undergone incubation of 24, 48, and 72 hours. Cells detachment was done by Trypsin-EDTA and cell pellet were collected via centrifugation at 1,500 rpm for 100 minutes. Cultured medium was then discarded and washed with PBS. Acridine orange and propidium iodide were used to stain the cells while fluorescence microscope was used to view the cells at 400x magnification.

RNase/PI staining

The CycleTEST™ Plus DNA Reagent Kit was used following the manufacturer's protocol (BD Biosciences, San Jose, CA, USA). The chosen cell lines were pre-incubated at the density of 1 x 10^6 cells/ml in 25 cm^2 flask for 24 hours and were treated with 40, 80, and 120 µg/ml of cashew shoot extract and incubated for 72 hours. Centrifugation of the cell has been done at 30 x g at room temperature for 5 minutes and subsequently washed with buffer solution for cell pelleting. 250 µl of solution A (trypsin buffer) and 200 µl of solution B (trypsin inhibitor and Rnase buffer) were added to cells and allowed it to react for 10 minutes at room temperature, respectively. The mixture is added with Cold solution C (200 µl of PI stain solution) and incubated for another 10 minutes at 4 °C. The cell filtration was done through a 35-nm cell strainer cap. Data collection and analysis were done by flow cytometry FACSCalibur™ Flow Cytometer with CellQuest 3.3 operating software (18).

Gas Chromatography Mass Spectrophotometry (GCMS)

The cashew shoot extract was subjected to GCMS analysis by using GCMS QP2010 Plus (Shimadzu, Kyoto, Japan) system. The system was equipped with SGE™ BPX5 GC capillary column, length: 30, internal diameter: 0.25 mm, film thickness: 0.25 µm. Helium was used as carrier gas for mobile phase and the flow rate was set at 1.0 ml/1 minute. The temperature programme of the oven was set at 50 °C/min and rise to 240 °C with a ramp of 5 °C/min and then increment of 10 °C/minute to final temperature of 280 °C, held for 15 minutes.. The National Institute Standard and Technology (NIST) NIST08 GCMS library database was used for the comparison for the spectral data (19).

Liquid Chromatography Mass Spectrometry (LCMS)

LCMS analysis of cashew shoot extract were performed by using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source equipped with Agilent Eclipse XDB-C18 Narrow-bore column (150 mm length x 2.1 mm internal diameter, 3.5-micron particle size) with column temperature at 25 °C, autosampler temperature at 4 °C, flowrate at 0.5 mL/mins. There are 2 types of solvents used which are 0.1 % formic acid in water as solvent A and 0.1 % formic acid in acetonitrile as solvent B according to Araujo et al. (2020). The injection volume was set at 1.0 uL. The gradient was start with 5 % B, then remained until 20 min and 25 min with 100 % B with post run time of 5 min. For MS condition, the source of ESI Ion polarity was set at negative and the mass range was from 100 – 3200 m/z. The raw data was processed with Molecular Feature Extraction with Extraction algorithm set at small molecule (chromatographic), peak filters with peak height ≥100 counts and compound filters to only look for compound with absolute height ≥5000 counts and relative height ≥2.5%. The data was processed with Agilent MassHunter Qualitative Analysis B.07.00 (20).

Statistical analysis

The statistical analysis of data was performed by using Statistical Package for Social Sciences (SPSS) version 19.0 (SPSS Inc., Chicago, USA). All experiments were performed in triplicates and data was expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test were performed for data analysis with $p<0.05$ were considered as statistically significant.

RESULTS

MTT Cytotoxic Assay

All the cell lines were treated with several concentrations of 70 % ethanolic cashew shoot extract range from 0 – 1000 µg/ml for 72 hours incubation. Fig. 1 demonstrated the dose-response curves of cashew shoots extract against MDA-MB-231, HT-29, HepG2 and 3T3 cells. The lowest IC_{50} value was shown in MDA-MB-231, followed by HT-29 and HepG2 (Table I). The lower IC_{50} value indicates higher cytotoxic effect thus having potential as anti-proliferative agents. This result showed its high cytotoxic effect for MDA-MB-231 while less

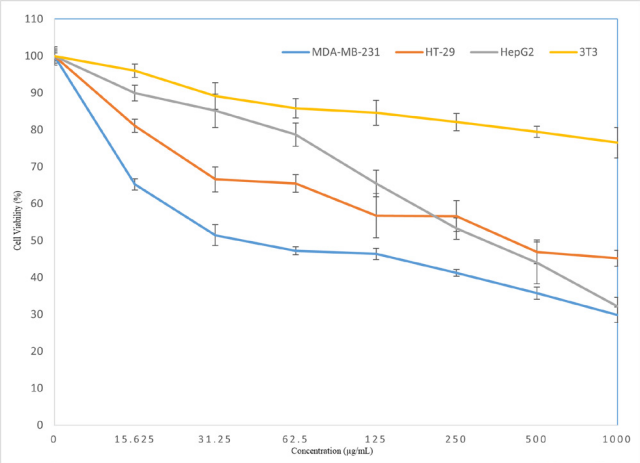

Figure 1: Dose-response curve of cashew shoot extract in (A) breast cancer (MDA-MB-231) cells, (B) colorectal cancer (HT-29), (C) liver cancer (HepG2) cells, and (D) normal mouse fibroblast (BALB/c 3T3) cells. The cell viability was evaluated by MTT assay after 72h incubation with cashew shoot extract of various concentrations (0 – 1000 µg/ml). The values reported in mean ± SD (n=3). MTT = 3-(4, 5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide.* indicate statistically significant at $p < 0.05$ between different cell lines.

Table 1: IC_{50} values of cashew shoot extract and doxorubicin in breast cancer (MDA-MB-231), liver cancer (HepG2), colorectal cancer (HT-29), and normal mouse fibroblast (BALB/c 3T3) cell lines

Cell lines	IC_{50} value (µg/ml)	
	Cashew shoots extract	Doxorubicin
MDA-MB-231	81.1 ± 0.11[a]	1.87 ± 0.66[a]
HT-29	272.6 ± 2.31[b]	1.93 ± 0.99[a]
HepG2	307.5 ± 1.91[c]	2.29 ± 0.34[a]
BALB/c 3T3	>1000[d]	1.96 ± 0.56[a]

Different superscript letter within the same column indicates statistically significant at $p < 0.05$

cytotoxic for HT-29 and HepG2 cells. Furthermore, current findings show no cytotoxic effect towards BALB/c 3T3 cells following the treatment by incubating with cashew shoot extract as the IC_{50} cannot be determined with more than 80% cell growth even at high dose. These findings suggest the selective cytotoxic effects of cashew shoot extract was only in cancer cells. On the other hand, dimethyl sulfoxide (DMSO) was used for cryopreservation of the cell culture and as the solvent for dissolving sample extract. However, DMSO can be cytotoxic as it can increase cell permeability (21). All cell lines showed 100% viability without the presence of sample treatment indicates the DMSO does not induce any cell cytotoxicity.

Besides, doxorubicin is a commercial chemotherapy drugs was adopted as positive control in this study. It was capable to induce toxicity in all the cell lines (Table I). However, it also demonstrated prominent cytotoxic effect in BALB/c 3T3 cells at IC_{50} value of 1.96 ± 0.56 µg/ml that indicates cytotoxicity towards normal cells.

AO/PI Staining

MDA-MB-231 with IC_{50} values (< 100 µg/ml) was chosen to proceed for further analysis. It was treated with cashew shoot extract at 80 µg/ml and undergone 24, 48 and 72 hours of incubation. According to Fig. 2, the dense green colour was observed in cell body after 24 and 48 hours of incubations as the signs of early apoptosis such as chromatin condensation and nuclear fragmentation in MDA-MB-231 cell lines. There were also cell blebbing and late apoptotic cells as shown in orange color can be observed in the cells after 48 hours of incubation with cashew shoot extract (Fig. 2C). The MDA-MB-231 cells had undergone the secondary necrosis where bright red color was emitted after 72 hours of incubation. For the control as untreated MDA-MB-231 cell lines in Fig. 2A, green colour with intact nucleus were observed as the signs of healthy cells (Fig, 2A).

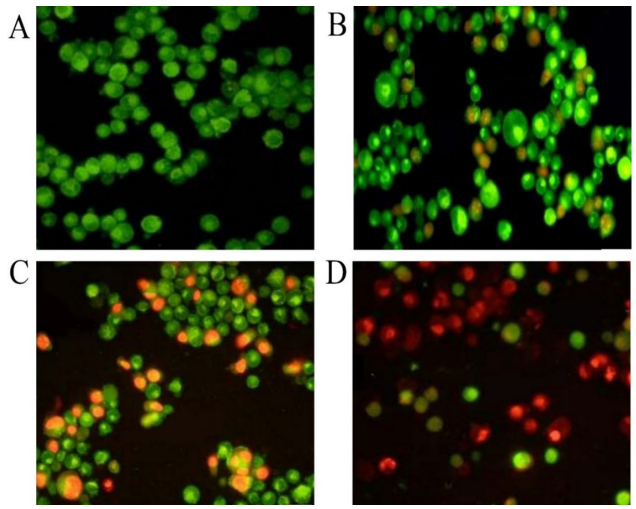

Figure 2: Morphological characterization of breast cancer (MDA-MB-231) cells after incubation with cashew shoot extract using acridine orang (AO) and propidium iodide (PI) staining. Breast cancer cell (MDA-MB-231) were either (A) untreated or treated with cashew shoots extract (80 µg/ml) after 24 h (B), 48 h (C) and 72 h (D) of incubations. Incubation of MDA-MB-231 cells with cashew shoot extract showed the characteristics of apoptosis such as chromatin condensation, nuclear fragmentation, and cell blebbing (Magnification 400 x).

RNase/PI staining

MDA-MB-231 cells were treated with three concentrations of cashew shoots extracts at 40, 80 and 120 µg/ml for 72 hours of incubation and showed in Fig. 3. According to Fig. 3, the cell number increased in sub-G0 phase with the incubation of cashew shoots extract compared with control. Treatment with cashew extract caused a significant increase in sub-G0 phase, indicative of cell death. This increase in sub-G0 cell population was accompanied with a significant decrease ($p<0.05$) in the population of cells in G0/G1, S and G2/M phase as shown in Fig. 3. The treatment at 40 µg/ml concentration increased 21.74 ± 4.52 % cells in sub-G0 phase, further increasing to 50.10 ± 3.78 % (80 µg/ml) and 76.32 ± 8.44 % at the highest concentration of 120 µg/ml compared to untreated cells (5.94%) ($p<0.05$), in a dose-dependent manner (Fig. 3).

Gas Chromatography Mass Spectrophotometry (GCMS) analysis

The GCMS analysis result of cashew shoot extract showed in Table II. There are 38 identified compounds which are composed primarily of sitosterol, pyrogallol, tannin and phenol (Table II).

Liquid Chromatography Mass Spectrometry (LCMS) analysis

Table III showed the identification of metabolites present cashew shoot. Based on the findings, there are 20 compounds identified in cashew shoot extract. Among

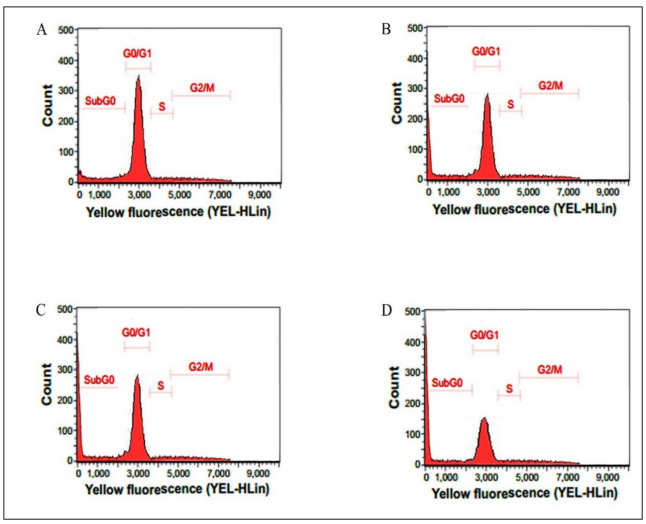

Figure 3: Flow cytometric of cell cycle analysis using RNase/PI staining of breast cancer (MDA-MB-231) after incubation with cashew shoot extract for 72h. Breast cancer cell (MDA-MB-231) were either untreated (A) or treated with cashew shoot extract at 40 µg/ml (B), 80 µg/ml (C) and 120 µg/ml (D) for 72 h. PI = Propidium iodide. The values were reported in mean ± SD (n=3).

Table II: Compounds that identified in cashew shoot extract using GCMS

PEAK	COMPOUND	Retention time	Area (%)
1	2-Hexanol	4.66	1.43
2	2,4-Dimethyl-1-heptene	4.99	0.09
3	trans-3-Hexen-1-ol	5.94	0.31
4	.alpha.-Pinene	7.71	0.26
5	Anthraquinone	8.72	0.28
6	Furan	9.97	0.27
7	1,3-Dioxolane	10.29	1.98
8	Lactic Acid	10.51	3.53
9	Beta-sitosterol	11.23	3.03
10	2-Furanmethanol	12.85	0.17
11	Phenol	14.96	13.09
12	Benzoic Acid	15.94	0.67
13	Catechol	16.78	2.99
14	Benzene	17.42	0.25
15	Phosphonic Acid	17.62	0.16
16	Phosphoric acid	17.73	0.24
17	Acetamide	19.23	0.97
18	Toluene	20.85	0.24
19	Copaene,	21.08	0.09
20	Sitosterol	21.58	20.65
21	Pyrogallol	21.72	18.72
22	Tannin	21.98	17.23
23	cis-Piperitol	22.79	0.99
24	1,3-Propanediol	23.29	2.51
25	Pentadecanoic acid	24.48	0.45
26	m-salicyclic acid	24.84	0.74
27	Asarone	26.07	0.92
28	Squalene	27.23	0.11
29	1-Deoxyhexitol	28.18	1.97
30	cis-Asarone	28.67	1.43
31	Inositol	31.63	0.38
32	Ascorbic Acid	34.46	0.98
33	Hexadecanoic acid	34.88	0.77
34	Phytol	37.06	0.71
35	Oleic Acid	37.84	0.29
36	9,12-Octadecadienoic acid	38.01	0.17
37	Linolenic acid	38.14	0.85
38	Octadecanoic acid	38.54	0.08

these compounds, only several compounds are reported with anti-cancer activity includes citric acid, gallic acid, myricetin and hinokiflavone.

DISCUSSION

Cashew shoot extract demonstrates anti-proliferation in all the studied cancer cell lines MDA-MB-231, HT-29 and HepG2 and distinctly higher cytotoxic effect against MDA-MB-231. Cashew extract has been previously studied of its cytotoxic effect toward other types of cancer cell lines. A study has been shown that the ethanolic cashew leaf extract had cytotoxic effect towards Jurkat cells (acute lymphoblastic leukemia) by apoptosis induction. The leaf extract used in the study was fractioned using high performance liquid chromatography (HPLC) where the biflavonoid agathisflavone was isolated and showed the IC_{50} value of 2.4 µg/ml towards Jurkat cells (22). Furthermore, 4 compounds isolated from cashew leaves namely zoapatanolide A, agathisflavone, anacardicin and methyl gallate reported cytotoxic activity for HeLa cells (23). Similarly, another study reported ethyl acetate leaves fraction of cashew leave exhibit cytotoxic activity on several cancer cell line. One of the bioactive components namely agasthisflavone was reported having better atomic contact energy compared to standard drug doxorubicin suggesting the probable mode of action on p38α MAP kinase that play a significant role in cancer

Table III: LC-ESI-MS/MS identification of metabolites present in 70% ethanolic extraction of *Anacardium Occidentale L.* Shoot.

Peak	Retention time tR (min)	Compound Name	Formula	Mass	m/z
1	0.635	D-mannonate	C6H12O7	196.0583	195.051
3	0.644	L-arabinonic	C5H10O6	166.0479	165.0406
6	0.667	L-Galactose	C6H12O6	180.0637	179.0565
7	0.697	Nifuradene	C8H8N4O4	224.0542	223.0468
8	0.711	Methylisocitric acid	C7H10O7	206.0433	205.0359
11	0.718	(R)-(Homo)2-citrate	C8H12O7	220.0564	219.0514
12	0.761	Citric Acid	C6H8O7	192.028	191.0201
13	1.512	Gallic Acid	C7H6O5	170.0215	169.0142
15	7.347	Digallate	C14H10O9	322.0325	321.0254
16	7.957	1,3,4-Trigalloyl-beta-D-glucopyranose	C27H24O18	636.097	635.0898
18	8.327	2-Hydroxy-3,4-dimethoxybenzoic Acid	C9H10O5	198.0524	197.0451
19	8.47	Myricetin	C21H20O13	480.0914	479.0836
22	8.891	8-Hydroxyluteolin 8-glucoside	C21H20O12	464.0961	463.089
24	9.09	Avicularin	C20H18O11	434.0857	433.0784
25	9.144	6-Hydroxyluteolin 5-rhamnoside	C21H20O11	448.1006	447.0934
33	9.901	Majusculoic acid	C15H23BrO2	314.0881	349.0575
34	11.664	Hinokiflavone	C30H18O10	538.0899	537.0831
36	15.178	2-O-(beta-D-galactopyranosyl-(1->6)-beta-D-galactopyranosyl) 2S-hydroxytridecanoic acid	C25H46O13	554.2921	553.2857
37	18.388	3α-Hydroxy-12-oxo-5β-chol-8-en-24-oic Acid	C24H36O4	388.263	387.2558
38	18.389	Cannabidiol dimethyl ether	C23H34O2	342.2569	341.2499

(24). Besides, there was another study also reported the cytotoxic activity of cashew leaf extract. The study investigated the cytotoxic effect in HepG2 cells by using methanolic extract of cashew leaf and it was showed that the IC_{50} value was 1.7 mg/ml (25). Compared with our current study, the IC_{50} value of cashew shoot ethanolic extract at 307.5 µg/ml was much lower compared to the IC_{50} value of methanolic extract in HepG2 cells showing ethanolic extraction is a better solvent for its cytotoxic effect in liver cancer cells, HepG2. Moreover, different solvent used in the extraction of samples may influence the types of compounds that contain in the extract.

Cytotoxic effect was not seen on BALB/c 3T3 cells following the incubation of cashew shoot extract for 72 hours as IC_{50} is unable to be determined with >80% cells growth. The selective index (SI) of cashew shoot extract towards non-cancerous cells 3T3 >1000 µg/ml (26). This finding demonstrated the selective anti-cancer properties of cashew shoot extract as cytotoxic effect was only shown in cancer cell lines but not in normal cell. This is an important feature of anticancer agent as to not affect normal cells to ensure that non-target cells are not affected by treatment and the side effects of the treatment can be minimized (27). Moreover, ethanolic cashew leaf extract also has been shown to caused only 22% and 26% mortality towards Chinese hamster lung fibroblast (V79) and human gingival fibroblast cell lines respectively (26), showing less toxicity towards normal cells.

Other parts of cashew plants (nut and fruit) have been investigated to study the apoptosis induction of the plants towards cancer cells. For instance, a study demonstrated the apoptosis mechanism of anacardic acid that extracted from cashew nut shell involving apoptosis-inducing factor and intrinsic pathway executioner that led to mitochondrial mediated apoptosis in liver cancer cells (HepG2) and lung cancer cells (A549) (28).

Besides, another study reported apoptosis induction through the increased of death receptors and decreased of anti-apoptotic proteins in cancer cell when anacardic acid synergize with TRAIL (29). Our present study showed the potential of apoptosis induction effect of cashew extract towards different types of cancer cells namely breast cancer cells as MDA-MB-231 cell lines. These are supported by AO/PI staining showing the early apoptotic cells (chromatin condensation, nuclear fragmentation, and cell blebbing) and late apoptotic cells that were observed in MDA-MB-231 and HT-29 cells incubated with cashew shoots extract. This indicates that the cell apoptosis that leads the splitting of nuclear DNA into several fragments (19,30).

This present study of GCMS analysis showed that cashew shoot ethanolic extract was mainly composed sitosterol, pyrogallol, tannin and phenol. Sitosterol is one of the plant sterols which provide major source of micronutrient and at same time shows no to any side effect to human (31). It has several medicinal properties that includes antioxidant, antidiabetic, anti-inflammatory and anti-cancer (32). Its anti-cancer effect mainly through promoting maglinant cell apoptosis, inhibiting proliferation of malignant cells and affect the cell cycle, which might explain the potential of this plant to cause apoptosis in cancer cell (33).

A previous study had reported that cashew shoot aqueous extract contains phytochemicals such as phenols, flavonoids, steroids, and triterpenes (34). Furthermore, there is another study found that major constituent of this plant methanolic extract consist of α-copaene, β-ocimene and δ-cadinene in addition of the 15 phenolic compounds. The phenolic compounds are mainly flavonol glycosides such as myricetin glycoside, Kaempferol-3-0-glucosides, quercetin-3-0-glucoside, and myricetin-3-0-rhamnosides (35).

Besides, tannins are natural polyphenolic compound that can be found abundantly in nature and present in many foods that origin from plants (36) was also abundant in cashew. Tannin has been reported to have anti-microbial effect towards yeast, fungi, bacteria, and virus (37). Since tannins was a polyphenol compound, it is reported that polyphenols that found in plants displays many anti-carcinogenic activities such as anti-proliferative effects on cancer cells, tumor growth, as well as apoptosis induction (38). Tannin reported to affect several oncological signaling pathways that can explain the anti-proliferative properties of cashew shoot extract against several cancer cell lines in this study (39). In addition, phenol also known as phenolic acids are aromatic secondary metabolites that can widely obtained from plants. Phenolic compounds have been reported cytotoxic effect towards cancer cells (40). Pyrogallol is a type of phenolic compound that present in oak and hardwood plants naturally. It is reported that pyrogallol possess anti-inflammatory effect towards chronic lung inflammatory disease (41). Other studies of pyrogallol isolated from Acacia nilotica reported anti-cancer activity against HT-29 cells lines and anti-colon cancer activity in mice model with less toxicity (42). There are 20 compounds identified in ethanolic cashew shoot extract in the present study. Among these compounds, only several compounds are reported with anticancer activity includes citric acid, gallic acid, myricetin and hinokiflavone.

Citric acid is an organic acid present in some fruits and vegetables juices, especially in citrus fruits. Citric acid has been distinguished in this study and also been discovered as the substantial organic acid found in cashew apples juice. It has been demonstrated that the citric acid accumulation repressed lung cancer cell proliferation and prostate cancer (43). Gallic acid is normally found in fruits and vegetables and thought as a potential anti-cancer, anti-inflammatory, antimicrobial,

immunoregulatory and antioxidant (44). Gallic acid has been reported in other part of cashew plant such as cashew flower, cashew apple juice and stem bark. Moreover, both ethanolic extract of cashew leaves and bark reported gallic acid content and demonstrated promising anti-cancer activity against several tumour cell lines (34). Myricetin is a type of polyphenol flavonoid that commonly found in fruits and vegetables. It has been reported with antioxidant, antiviral, antimicrobial, cytoprotective and antiplatelet properties (45). Myricetin has been reported anti-cancer properties in various types of cancers such as breast cancer, colorectal cancer and liver cancer cell (46). Hinokiflavone is a type of bioflavonoid that are present in plants and reported in one of the fractionations in crude ethanolic cashew leaves extract that contributed to its anti-proliferative effect (47). Hinokiflavone has also demonstrated its selective anti-proliferation efficacy in liver cancer cell lines of SMMC-7721 and HepG2 while having low cytotoxicity towards normal hepatocyte (48).

CONCLUSION

In this present study, cashew shoot extract has demonstrated its cytotoxic effect against breast cancer, MDA-MB-231 cells with IC_{50} of 81.1 µg/ml and less cytotoxic for HT-29 and HepG2 with IC_{50} value of 272.6 and 307.5 µg/ml respectively. In addition, it was also demonstrated that cashew shoots extracts had no or toxic effects in mouse fibroblast cell line (BALB/c 3T3). This implies that cashew extracts are selective anti-proliferative agents towards cancer cells while not affecting normal cells. Furthermore, it was shown that cashew shoot extract is able to induce apoptosis in MDA-MB-231 cell lines. Cashew shoot extract was found to contain various phytochemicals such as sitosterol, tannin, pyrogallol and phenol from GCMS analysis, citric acid, gallic acid, myricetin and hinokiflavone from LCMS analysis that may give rise to its anti-cancer effect. Further studies of the identified compounds in cashew shoot extract can be done for their anti-cancer properties. From this study, it can be concluded that cashew shoots extracts possess anti-cancer effects towards chosen cancer cells through apoptosis induction. More study is needed to obtain further information about cashew plant as one of the functional foods as an alternative for cancer therapy.

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