Al-Rafidain J Med Sci. 2024;7(2):101-108.
DOI: https://doi.org/10.54133/ajms.v7i2.1373

A J M S
Online ISSN (2789-3219)

Research Article

The Free Radical Scavenging and Cytotoxic Properties of *Ardisia crenata* and *Peliosanthes teta* in the 4T1 Breast Cancer Cell Line

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Received: 20 September 2024; Revised: 8 November 2024; Accepted: 19 November 2024

Abstract

Background: *Ardisia crenata* (*A. crenata*) and *Peliosanthes teta* Andrew (*P. teta*) are popular medicinal plants in Malaysia that are claimed to have several biological effects. ***Objective***: To assess the anticancer and radical-scavenging properties of *A. crenata* and *P. teta* methanolic extracts. Methods: TPC was quantified using the Folin-Ciocalteu test; free radical scavenging was evaluated using the 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays; and cytotoxic activity was assessed using the MTT assay. ***Results***: In both ABTS and DPPH systems, A. crenata exhibits much higher radical scavenging activity than ascorbic acid. *P. teta* showed less significant antioxidant activity. Furthermore, *A. crenata* methanolic extracts demonstrated free radical scavenging properties in a concentration-dependent manner, quenching ABTS and DPPH radicals with no lag phase observed in the experiments. Furthermore, *A. crenata* had a strong cytotoxic effect on the breast cancer 4T1 cell line but had no suppression of cell viability in the 3T3 normal fibroblast cell line. Interestingly, the TPC of *A. crenata* was significantly higher than that of *P. teta* methanolic extracts. ***Conclusions***: *A. crenata* showed antioxidant and anticancer action against breast cancer 4T1 cells, indicating that it could be a valuable source for future pharmacological and medical research.

Keywords: Antioxidant activity, Anticancer activity, *Ardisia crenata*, Kinetic reaction, *Peliosanthes teta*; Total antioxidant capacity.

خصائص قنص الجذور الحرة وسمية الخلايا ل *Ardisia crenata* و *Peliosanthes teta* في خط خلايا سرطان الثدي 4T1

الخلاصة

الخلفية: *Ardisia crenata* (*A. crenata*) و *Peliosanthes teta* Andrew (*P. teta*) هي نباتات طبية شهيرة في ماليزيا يزعم أن لها العديد من التأثيرات البيولوجية. **الهدف**: تقييم الخصائص المضادة للسرطان والكسح الجذري لمستخلصات *المیثانول A. crenata* و *P. teta*. **الطرق**: تم قياس TPC باستخدام اختبار Folin-Ciocalteu. تم تقييم كسح الجذور الحرة باستخدام فحوصات 2،2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) و 2،2-diphenyl-1-picrylhydrazyl (DPPH). وتم تقييم النشاط السام للخلايا باستخدام مقايسة MTT. **النتائج**: في كل من أنظمة ABTS و A. crenata ، DPPH يظهر نشاط كسح جذري أعلى بكثير من حمض الأسكوربيك. *أظهر P. teta* نشاطا أقل أهمية مضادا للأكسدة. علاوة على ذلك، *A. crenata* أظهرت مستخلصات الميثانول خصائص كسح الجذور الحرة بطريقة تعتمد على التركيز ، مما يؤدي إلى إخماد جذور ABTS و DPPH دون ملاحظة أي مرحلة تأخر في التجارب. علاوة على ذلك ، *A. crenata* كان له تأثير قوي سام للخلايا على خط خلايا سرطان الثدي 4T1 ولكن لم يكن لديه قمع لصلاحية الخلية في خط الخلايا الليفية الطبيعية 3T3. ومن المثير للاهتمام أن TPC ل *A. crenata* كان أعلى بكثير من مستخلصات *المیثانول P. teta*. **الاستنتاجات**: *أظهر A. crenata* تأثيرا مضادا للأكسدة ومضادا للسرطان ضد خلايا سرطان الثدي 4T1، مما يشير إلى أنه يمكن أن يكون مصدرا قيما للأبحاث الدوائية والطبية في المستقبل.

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Article citation: Al-Zubaidi YMA, Abdul Kadir A, Zakaria ZA. The Free Radical Scavenging and Cytotoxic Properties of *Ardisia crenata* and *Peliosanthes teta* in the 4T1 Breast Cancer Cell Line. *Al-Rafidain J Med Sci*. 2024;7(2):101-108. doi: https://doi.org/10.54133/ajms.v7i2.1373

INTRODUCTION

In recent years, there has been significant attention given to oxidative stress in the body. This condition is defined as an increase in oxidant production in living cells, characterized by the creation of free radicals that cause numerous cellular oxidative damages [1,2]. Numerous studies have linked oxidative stress to diseases such as cancer, diabetes, atherosclerosis, and osteoarthritis [3–7]. The generation of free radicals may occur as part of normal physiological processes or because of pathogenesis. They can be very harmful and cause damage to lipids, proteins, DNA, and carbohydrates.

As a result of this damage, normal cell function will be deteriorated with the consequence of disease and eventually death [8–10]. There are, however, antioxidant defense mechanisms in the body that assist in preventing or scavenging oxidative stress caused by free radicals. The body's antioxidant defense could be provided by enzymes like catalase, superoxide dismutase, and glutathione peroxidase, as well as non-enzymatic antioxidant molecules like vitamins, carotenoids, phenols, and flavonoids [11–14]. By enhancing the body's enzymatic antioxidant defense or supplementing with proven antioxidants, many diseases caused by oxidative stress, such as cancer, can be reduced. In this context, finding natural antioxidants has become a focus. *Ardisia crenata* is a species of *Myrsinaceae* found naturally in Malaysia, Indonesia, southern China, southeast Asia, and the Philippines as a forest understory plant. It is an evergreen shrub that grows up to 2 meters tall, with thick, dark green, glossy leaves. A traditional medicine has used this plant for treating respiratory tract infections and menstrual disorders [15]. Additionally, a previous study reported significant antitumor effects against melanoma WM793 and colon cancer Caco2 [16]. *Peliosanthes teta Andrew*, commonly known as Bujang Hilir, is another popular medicinal plant from the *Convallariaceae* family. It is native to Malaysia, India, Laos, Myanmar, and Thailand. The plant is used by natives as an aphrodisiac and a postnatal tonic in folk medicine [17]. However, despite their widespread usage, these plants have not been sufficiently studied for their antioxidant and anti-cancer properties. In this study, the antioxidant and anticancer activities of the plants' extracts were evaluated. Furthermore, the total phenolic compounds present in these plants were also assessed.

METHODS

Plant materials

The Unit of Biodiversity, Institute of Bioscience (IBS), University Putra Malaysia (UPM), Malaysia's Assistant Agriculture Officer, collected and characterized both the mature leaves of *A. crenata* and *P. teta*. For every plant, a voucher specimen was placed in the IBS, UPM herbarium. The leaves of each plant (2 kg) were cleaned, dried, ground, and sieved through a 60-mesh before being stored at -20°C in an airtight plastic container until needed again.

Extraction procedure

For three days, the pulverized leaves of A. crenata and P. teta were extracted (cold extraction) using 99.85% methanol at a ratio of 1:20 (w/v). The extracts were filtered using Whatman #1 filter paper. To obtain the A. crenata and P. teta extracts, the filtrates were dried in a rotary evaporator at a controlled temperature (40–50 °C) and reduced pressure. After that, they were all kept at -20°C until used.

Total polyphenol assay

The Folin-Ciocalteu test was utilized to determine the total phenolic contents (TPC) of the ground leaf extracts of *A. crenata* and *P. teta*. A mixture of 750 µL diluted Folin-Ciocalteu reagent (10 folds) and 100 µL methanolic extract was prepared. Then, 0.75 mL of 6% (w/v) sodium carbonate solution was added. The TPC was measured at 725 nm using a Spectronic Genesis TM spectrophotometer. Gallic acid was used to plot the standard calibration curve at various doses (20–100 µg/mL). The TPC was expressed as mg/g of crude extract corresponding to gallic acid [18,19].

ABTS radical scavenging assay

The procedure described by Re *et al*. [20] was used to perform the ABTS experiment. Potassium persulfate (Sigma Chemical Co., USA) was added to the dissolved ABTS (Sigma Chemical Co., USA) at a concentration of 7.45 mM in deionized water. After that, 1 mL of the blue green ABTS radical solution was diluted with 20 mL of methanol to get an absorbance of 1.1±0.02 at 734 nm. Following that, 100 µL of diluted ABTS free radicals were added to 100 µL of *A. crenata*, *P. teta*, ascorbic acid, and butylated hydroxytoluene (BHT) on a 96-well microplate at different concentrations (in methanol). The control included 100 µL of methanol and 100 µL of ABTS free radicals. A microplate reader (ASYS UVM 340, Austria) was used to measure the absorbance at 734 nm. Fresh ABTS free radical stock solution was made daily, and each sample underwent at least three replications of analysis.

DPPH radical scavenging assay

The DPPH assay was performed using a method that was developed by She *et al*. [21], with slight modifications. A 500 µM solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical was generated by dissolving DPPH (Sigma Chemical Co., USA) in methanol (Sigma Chemical Co., USA) and shaking vigorously. A sample reaction well with 100 µL of *A. crenata*, *P. teta*, ascorbic acid, and BHT was mixed with 100 µL of 500 µM DPPH free radical solution. The addition of a DPPH free radical solution to each sample examined in a microplate triggered the reaction between the antioxidant and the DPPH radical. Absorption was measured at 517 nm immediately following the addition of DPPH free radical solution to the microplate wells. An absorption reading of 517 nm was measured for a mixture of 100 µL of solvent (methanol) and 100 µL of 500 µM DPPH-free radical solutions as a control. All samples were analyzed at least three times, and

DPPH free radical stock solution was made fresh every day.

Median inhibitory concentration (IC_{50}) quantification

The median inhibitory concentration (IC50) is the amount of the antioxidant that stops the production of ABTS or DPPH radicals by half in a test setting [22]. The IC_{50} of *A. crenata*, *P. teta*, ascorbic acid, and BHT were calculated by graphing the percentage ABTS or DPPH radical remaining at steady state of the reaction versus the concentration of the investigated compounds. The IC_{50} values were then calculated from the concentration-response curves for each investigated sample using formula 1.

$$(50\% - A\%)/(B\% - 50\%) = (X - CA)/(CB - X) \text{ ------- 1.}$$

Where A%= the % inhibition immediately above 50%; B%= the % inhibition immediately below 50%; CA= the concentration with the % inhibition immediately above 50%; CB= the concentration with the % inhibition immediately below 50%; and X= IC_{50} value.

Kinetic reactions

The kinetic reactions of a methanolic extract of A. crenata against ABTS or DPPH radicals in methanol were measured by monitoring the radical disappearances in the reaction mixtures using a method developed by Yu [23]. The kinetic investigation used A. crenata extract at six concentrations (10, 20, 40, 80, 160, and 320 µg/mL methanol). A mixture of 100 µL of the ABTS or DPPH methanol solution was combined with 100 µL of each extract solution. To demonstrate kinetic properties, each extract's reaction was obtained by graphing the remaining amount of ABTS or DPPH free radical against time at each level. The values of % ABTS or % DPPH free radical remaining at different reaction periods for various amounts of each extract were obtained using formula 2.

$$\%\ \text{free radical} = ((\text{A sample})/(\text{A control})) * 100 \text{ ------- 2.}$$

Where A sample = absorbance of the certain concentration of a selected antioxidant measured at certain reaction time, and A control = absorbance of the control measured at certain reaction time.

Total antioxidant capacity quantification

Using ABTS and DPPH free radicals, the total antioxidant capacity (TAC) of *A. crenata* was calculated and compared to ascorbic acid and BHT using a technique by Winston *et al*. [24]. To initiate the reaction, 100 µL of ABTS or DPPH radical solutions were mixed with 100 µL of methanolic solutions of *A. crenata*, ascorbic acid, and BHT. The final concentration for each test compound was 20 µg/mL, with the exception of the control containing pure methanol [23,25]. The area under the kinetic curve for each tested sample and control against time was obtained using integration. We then calculated the total antioxidant capacity (TAC) using formula 3.

$$TAC = 100 - ((\int SA)/(\int CA) * 100) \text{ ------ 3.}$$

Where $\int$ SA = the integrated areas of the curve defining the sample reactions, and $\int$ CA = the integrated areas of the curve defining the control reactions.

Cell viability assay

In this study, *A. crenata* and *P. teta's* anticancer activity was evaluated using a triple-negative mouse breast cancer cell line 4T1 and a normal embryonic mouse fibroblast cell line 3T3 from the American Type Culture Collection (ATCC). The cells were mycoplasma-free and were grown in RPMI medium with 10% FBS and 1% penicillin/streptomycin [26]. The MTT assay was performed to determine cell viability, as previously described [27–29]. The cells were seeded in 96-well plates (5×10^3/well) and incubated for 24 hours at 5% CO_2 and 37°C. The cultured cells were then treated with test extracts while the control cells were treated with the same amount of solvent (DMSO, 0.1% final), and both were incubated for 48 hours before MTT (62.5 µg/25 µL) was added. Plates were incubated at 37°C for 2 hours, then 100 µL of DMSO was added to dissolve the formazan product. The absorbance was measured at 540 nm using a Victor 3 V 1420 multi-label counter (Perkin Elmer, Akron, OH, USA).

Statistical analysis

Data were presented as mean±SD in the antioxidant results and mean±SEM in the anticancer results. Differences among the mean (n=3) of tested samples were assessed using the T test for two groups and one-way analysis of variance (ANOVA) for more than two groups after verification of the normal distribution of the data, followed by a Tukey *post hoc* test. The SPSS 15 software was used to conduct all statistical tests (SPSS, 2006). At $p< 0.05$, significant differences were considered.

RESULTS

Table 1 shows the TPC, voucher numbers, and yield of methanolic extract obtained from plants with scientific and common names. The yield from the crud materials of *A. crenata* methanolic extract was higher than that of *P. teta* methanolic extract. Furthermore, *A. crenata* methanolic extract had significantly higher TPC ($p< 0.05$) than *P. teta* methanolic extract (116.7±7.5 and 47.6±4.3, respectively). In this study, the inhibition percent of ABTS and DPPH free radicals at steady state against the corresponding concentrations of methanolic

extracts of *A. crenata* and *P. teta* with standard antioxidants ascorbic acid and BHT are shown in Figure 1 (A and B).

Table 1: The total polyphenolic contents, voucher numbers and the yield of methanolic extracts obtained from the plants with scientific name and common name

Scientific Name	Common Name	Voucher No	Yield (%)	Total Polyphenolics mg GAE/g ± SD
Ardisia Crenata (Roxb)	Mata Ayam	ACP 0107	23.78	116.7 ± 7.5
Peliosanthes Teta Andrews ssp. teta	Bujang Hilir	ACP 0096	18.5	47.6± 4.3

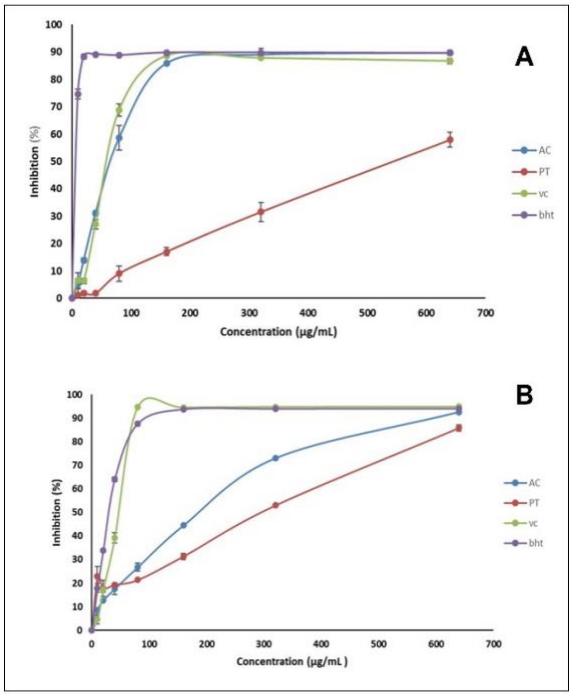

Figure 1: A) Disappearance of ABTS as a function of *A. crenata* (AC), *P. teta* (PT), ascorbic acid (VC) and butylated hydroxytoluene (BHT) concentration at steady state of reaction. B) Disappearance of DPPH as a function of *A. crenata* (AC), *P. teta* (PT), ascorbic acid (VC) and butylated hydroxytoluene (BHT) concentration at steady state of reaction. Data are expressed as mean±SD (n=3).

The scavenging activity of both extracts against ABTS and DPPH free radicals was concentration dependent. The IC_{50} values of all tested samples through the ABTS free radical system ranged from 6 to 543 µg/mL, and ABTS scavenging activity is arranged in the following descending order: BHT, ascorbic acid, *A. crenata*, and *P. teta*, respectively. At a low concentration (10 µg/mL), *A. crenata* extract quenched comparable ABTS radicals to that of ascorbic acid. Additionally, the ABTS scavenging activity of *A. crenata* extract was comparable to the standard antioxidants BHT and ascorbic acid at high concentrations (160 and 320 µg/mL). While at concentrations of 20 and 40 µg/mL, *A. crenata* extract quenched significantly ($p< 0.05$) higher ABTS radicals in comparison to that of ascorbic acid (Table 2). On the other hand, DPPH assay revealed that IC_{50} values of all tested samples ranging from 30 to 297 µg/mL, arranged in the following descending order: BHT > ascorbic acid > *A. crenata* > *P. teta*. The extract of *A. crenata* exhibited a higher ($p< 0.05$) DPPH free radical scavenging activity than that of ascorbic acid at a low concentration (10 µg/mL) and was comparable to ascorbic acid at 20 and 320 µg/mL (Table 3).

Table 2: Inhibition % of ABTS radical at steady state among different concentrations of tested extracts, and median inhibitory concentration (IC_{50}) value

Tested Compound	Concentration (µg/mL) ABTS						
	10	20	40	80	160	320	IC_{50}
Ardisia Crenata (Roxb)	5.17±1.7^a	13.9±0.7^a	31.1±0.7^a	58.6±4.5^a	85.8±0.3^a	89±0.83^a	67.9±4.3^a
Peliosanthes Teta Andrews ssp. teta	1.3±0.5^b	1.8±0.7^b	1.7±0.6^b	9±2.8^b	17±1.4^b	31.5±3.5^b	543.4±34^b
BHT	74.5±1.7^c	88.3±0.7^c	89.1±0.2^c	88.8±0.5^c	89.7±0.7^a	89.7±1.5^a	6±0.59^c
Ascorbic acid	6.5±2.7^a	6.3±1^d	27±1.6^d	68.7±2.1^a	88.5±0.4^a	87.8±0.4^a	62 ±0.8^a

Values were expressed as mean±SD (n=3). The median inhibitory (IC_{50}) value was defined as the concentration that caused 50% inhibition of ABTS radical formation at steady state of reaction. Values with different superscripts (a,b,c) among different compounds were significantly different ($p< 0.05$).

Table 3: Inhibition % of DPPH radical at steady state among different concentrations of tested extracts, and median inhibitory concentration (IC_{50}) value

Tested Compound	DPPH Concentration (µg/mL)						
	10	20	40	80	160	320	IC_{50}
Ardisia crenata (Roxb)	12.6±0.4^a	17.5±2.3^a	26.7±1.7^a	44.5±0.6^a	73±0.6^a	92.6±0.07^a	190.6±2.2^a
Peliosanthes teta Andrews ssp. teta	22.8±4.0^b	17.8±3.3^a	19.3±0.49^b	21.3±0.3^b	31±0.4^b	52.9±0.5^b	297.8±3.1^b
BHT	17.8±1.7^b	33.8±0.7^b	64±1^c	87.5±0.9^c	93.7±0.07^c	93.9±0.03^a	30±0.5^c
Ascorbic acid	4.6±1.8^c	17.3±1.2^a	39.2±2.1^d	94.6±0.005^d	94.5±0.2^c	94.8±0.03^a	47±1.2^d

Values were expressed as mean±SD (n=3). IC_{50} value was defined as the concentration that caused 50% inhibition of DPPH radical formation at steady state of reaction. Values with different superscripts (a,b,c,d) among different compounds were significantly different ($p< 0.05$).

A. crenata was further tested for the total antioxidant capacity (TAC) and compared with widely used antioxidants ascorbic acid and BHT by using ABTS and DPPH assays (Figure 2 A and B). The TAC

against ABTS free radical of *A. crenata* extract was observed to have a noticeable TAC; however, it was not as high as ascorbic acid and BHT at the same concentration of 20 µg/mL (15.9±1.6 %, 33.8±0.8%, and 82.7±1%, respectively). Meanwhile, the DPPH assay revealed a comparable TAC of *A. crenata* extract to that of ascorbic acid (6.18±0.57%) and 8.8±1.8%, respectively. However, they were less effective radical scavengers as compared to BHT (31±2.1%) at the same concentration in the DPPH system. Figure 3 (A and B) illustrates the kinetic reactions of *A. crenata* extract against DPPH and ABTS free radicals in methanol.

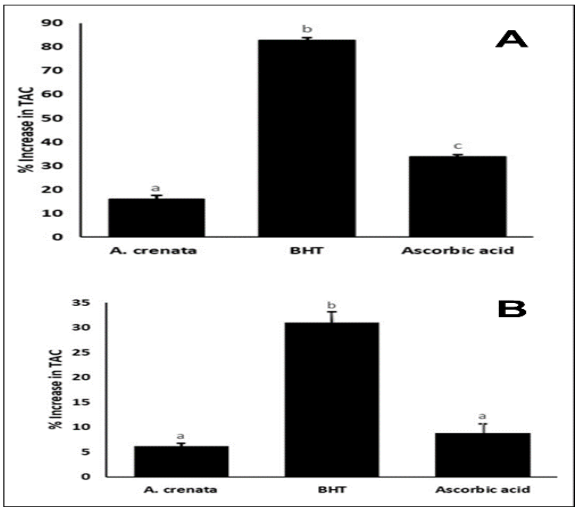

Figure 2: Comparison of total antioxidant capacity (TAC) of *A. crenata*, ascorbic acid and butylated hydroxytoluene (BHT). A) All antioxidants were compared at 20 µg/mL final concentration against ABTS free radicals. B) All antioxidants were compared at 20 µg/mL final concentration against DPPH free radicals. Data are presented as mean±SD (n=3). Columns marked with the same alphabet are not significantly different ($p< 0.05$).

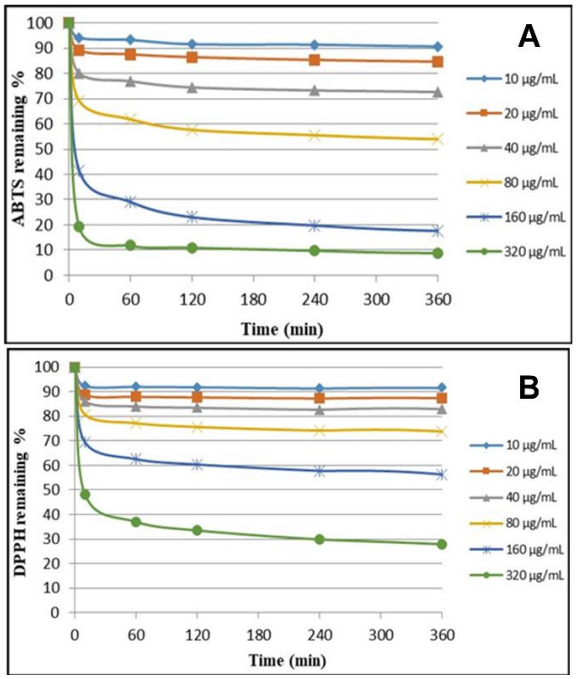

Figure 3: **A**) Kinetic curve of *A. crenata* against ABTS free radical. **B**) Kinetic curve of *A. crenata* against DPPH free radical. The final concentrations of tested extracts were 10, 20, 40, 80, 160 and 320 (µg/mL) in the reaction mixtures. All tests were conducted in triplicate and mean values are used.

The *A. crenata* extract demonstrated free radical scavenging action at all investigated concentrations, with the lowest concentration being 10 µg/mL in methanol. Concentration and time dependence were observed under the same testing conditions. Under the same conditions, more ABTS and DPPH free radicals were quenched with *A. crenata* extract at a greater 320 µg/mL concentration than other tested concentrations. *A. crenata* extract immediately reacted and quenched ABTS and DPPH free radicals at all tested concentrations. In addition, no lag phase was detected for all tested concentrations against free radicals in the ABTS and DPPH systems. In this work, the extracts of *A. crenata* and *P. teta* were also examined for anticancer activities. Figures 4 and 5 show the cytotoxicity effects of both plant extracts on the breast cancer 4T1 cell line.

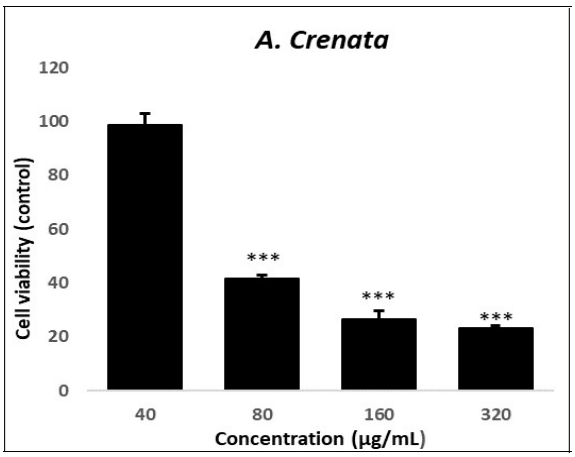

Figure 4: Cytotoxic effect of *A. crenata* methanolic extracts against 4T1 breast cancer cells. Different from corresponding control: ***$p< 0.001$. Data are presented as mean±SEM. n = 3 independent experiments.

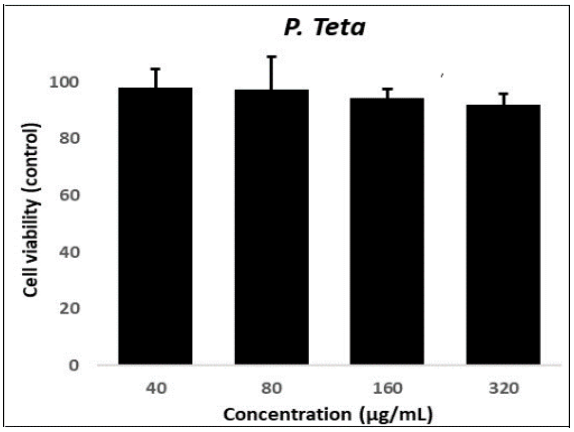

Figure 5: Cytotoxic effect of *P. teta* methanolic extracts against 4T1 breast cancer cells. Different from corresponding control: ***$p< 0.001$. Data are presented as mean±SEM. n= 3 independent experiments.

The anti-cancer activity of the investigated extracts was determined using the MTT assay. Figure 6 shows the reduction in MTT activities of *A. crenata* and *P. teta* methanolic extracts against 4T1 breast cancer cells. In a concentration-dependent manner, *A. crenata* significantly reduced the viability of 4T1 cells (IC_{50} = 74.1±0.64 µg/mL). Meanwhile, even at a high concentration of 320 µg/mL, *P. teta* had no

action against 4T1 cells. Thus, *A. crenata* was further tested against the 3T3 normal fibroblast cell line (Figure 6). It produced a minor inhibition of cell viability in the 3T3 normal fibroblast cell line when tested at 40, 80, and 160 µg/mL. However, at a higher concentration of 320 µg/mL, it showed a significant inhibition of 3T3 normal fibroblast cell viability (59.3±5.1%).

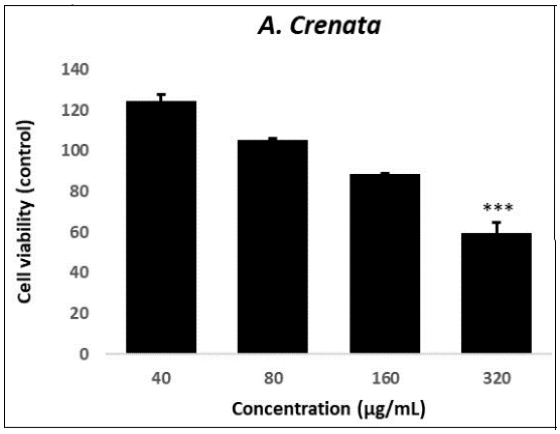

Figure 6: Cytotoxic effect of *A. crenata* methanolic extract against 3T3 normal fibroblast cell line. Data are presented as mean±SEM. n= 3 independent experiments.

DISCUSSION

Medicinal plants have therapeutic effects due to the fact they contain diverse phytotherapeutics, such as phenolic chemicals. They are considered the most important class of phytochemicals in plant extracts. According to numerous studies, phenolic compounds have been linked to antioxidant activity and the prevention of lipid peroxidation [30–32]. This is most likely due to their high scavenging abilities, which are attributed to their hydroxyl groups. Besides being potential antioxidants, polyphenolic compounds are thought to suppress tumors and carcinogenesis in humans [33,34]. In this study, *A. crenata* methanolic extract had significantly greater TPC than *P. teta* methanolic extract. Moreover, the *in vitro* antioxidant activities of *A. crenata* and *P. teta* extracts were evaluated on their scavenging ability on the stable ABTS and DPPH free radicals, which are the most used methods to determine antioxidant activity in plant extracts [25,35,36]. Comparison of ABTS and DPPH scavenging activities of the plant extracts with those expressed by the reference standard confirmed that *A. crenata* extract has a potential antioxidant property in comparison to ascorbic acid since it has been used as a reference standard for comparing plant extracts with potential antioxidants [37]. Furthermore, no lag phase was detected for all tested concentrations against free radicals in the ABTS and DPPH systems. The lag phase indicates the delay of radical quenching actions [38]. These findings suggest that *A. crenata* extract could provide immediate protection against free radicals. On the other hand, the anticancer activity of plant extracts needs to be evaluated to ensure safe treatment. It allows for the detection of the plant's intrinsic toxicity as well as the effects of acute overdose [39]. Hence, the extracts of *A. crenata* and *P. teta* were also examined for their anticancer activities against the breast cancer 4T1 cell line using the MTT assay. It is a widely used assay to evaluate the viability of cells by reducing the yellow tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a purple water-insoluble formazan in the presence of intact mitochondria [40,41]. The extract of *A. crenata* exhibited strong cytotoxicity against 4T1 breast cancer cells, which was concentration dependent. Additionally, at 40, 80, and 160 µg/mL, it caused a slight decrease of cell viability in the 3T3 normal fibroblast cell line. While the viability of 3T3 normal fibroblast cells was notably reduced to 320 µg/mL. This indicates that at the IC_{50} concentration, *A. crenata* exhibits preferential cytotoxicity towards cancer cells but not normal cells. Altogether, the reduction of 4T1 cell growth by *A. crenata* extract could be attributed to the influence of various bioactive components, phenolic compounds, and other antioxidant agents found in *A. crenata*.

Conclusion

The methanolic extract of *A. crenata* possessed high antioxidant and anticancer properties. Given their wide and extensive traditional uses and prospective pharmaceutical applications, further phytochemical and pharmacological research into *A. crenata* active substances is warranted.

ACKNOWLEDGMENTS

The authors thank the Unit of Biodiversity, Institute of Bioscience (IBS), Faculty of Medicine and Health Sciences and Faculty of Veterinary Medicine, University Putra Malaysia (UPM), Malaysia, for providing the facilities and support for the completion of this study.

Conflict of interests

No conflict of interest was declared by the authors.

Funding source

This project was supported by the Research University Grant Scheme (RUGS), Universiti Putra Malaysia (UPM), Malaysia (Grant Code: 01-02-12-2282RU).

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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