ORIGINAL ARTICLE

Preliminary Screening of *Durio Zibethinus* Linn (D197) Leaf Extracts for Its Antioxidant Activity and Cytotoxicity on Cervix Adenocarcinoma (HELA) Cancer Cell Line

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ABSTRACT

Introduction: Medicinal plants have always been in the spotlight of drug discoveries attributing to their effectiveness and minimal side effects. Durio zibethinus Linn (D197) leaves stand out with decent advantageous therapeutic effects apart from abundantly employed in traditional treatment. The purpose of the current study is to evaluate the antioxidant activity, total phenolic and total flavonoid contents as well as the cytotoxicity of Durio zibethinus Linn (D197) leaf extracts. **Methods:** Extraction of the leaves was performed using hexane, ethyl acetate, methanol and 70% aqueous methanol respectively via maceration. Extracts were screened for antioxidant potential using DPPH Free Radical Scavenging Activity, total phenolic content using Folin-Ciocalteu Assay, total flavonoid content using aluminium chloride colourimetric method, and cytotoxic properties on cervix adenocarcinoma (HeLa) cell line using MTT Assay. **Results:** Methanol demonstrated the highest percentage of extraction yield (2.73%) and the highest potency in DPPH free radical scavenging with EC_{50} value of 304.29 µg/mL followed by aqueous methanol (441.25 µg/mL), ethyl acetate (556.71 µg/mL) and hexane (>600 µg/mL). Highest effectiveness in phenolic compounds extraction was demonstrated by methanol (141.03 µg GAE/mg) followed by aqueous methanol (63.08 µg GAE/mg), ethyl acetate (41.79 µg GAE/mg) and hexane (36.92 µg GAE/mg). As for the total flavonoid content, high effectiveness of flavonoid extraction was exhibited by ethyl acetate (166.19 µg QE/mg) as compared to hexane (94.76 µg QE/mg), methanol (17.62 µg QE/mg) and aqueous methanol (13.81 µg QE/mg). Ethyl acetate emerged as the most potent extract in inhibiting HeLa cells with IC50 values of 19.95 µg/mL, 30.07 µg/mL and 23.42 µg/mL for 24, 48 and 72 hours respectively. **Conclusion:** *Durio zibethinus* Linn (D197) leaf extracts showed antioxidant and cytotoxic activities and thus, further studies are essential for development of possible cancer treatment.
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INTRODUCTION

The burden of chronic diseases such as cancers, atherosclerosis and diabetes are increasing rapidly around the globe. These chronic diseases are linked to oxidative stress resultant of reactive free radicals such as superoxide, hydroxyl and peroxyl radicals. As a result, the deleterious radicals can impose injuries and damage to tissues due to protein degradation, lipid peroxidation and oxidation of DNA [1, 2].

Natural products particularly medicinal plants have been in the spotlights of drug discoveries attributing to their wide array of chemical compositions that enable versatile applications [3]. The minimal adverse effects and broad effectiveness delivered by traditional plant medicine are also plausible despite their lower potency of action when compared to chemically synthesized drugs. Better still, the effectiveness of plant-based drugs can be enhanced via fusion treatment with other drugs [4, 5].

The therapeutic effects of plant products are accounted to the presence of bioactive compounds notably secondary metabolites. Secondary metabollites can be classified into three main classes namely terpenes and terpenoids, alkaloids and phenolic compounds [6]. These chemically diverse compounds hold variable crucial health protecting effects apart from advantageous biological activities such as antioxidant, antimicrobial, anticancer, anti-diabetic and reduction of both inflammation and mutagenesis in cells [7].

One of the plants that stands out to possess decent advantageous therapeutic effects is the leaves of Durio zibethinus Linn. The leaf of Durio zibethinus Linn (D197) or more commonly known as Musang King durian is pale or light bronze green in colour with shiny upper leaf surface and brownish lower surface which is covered with scales. With a length of 12 to 22 cm and width of 4 to 8 cm, the evergreen leaves are oblong lanceolate in shape, have a smooth leaf margin and an acuminate tip [8,9].

Some of the major phytochemical constituents detectable from the leaves of durian tree include tannins and saponins which are reported to exert antioxidant and anticancer properties [10,11]. Apart from that, durian leaves are utilized in abundant traditional treatments which further emphasize its therapeutic effects on human biological system. Among the roles of durian leaves extract as an age-old remedial include to serve as febrifuge or antipyretic, to alleviate colds and phlegm, to cure jaundice and to treat skin problems as well as swellings [12].

Thus, this present research was conducted to investigate the antioxidant activity, total phenolic and total flavonoid contents as well as cytotoxicity of different solvent extracted Durio zibethinus Linn (D197) leaf extracts through various biochemical assays and on human cervix adenocarcinoma (HeLa) cancer cells respectively.

MATERIALS AND METHODS

Chemicals and solvents

Hexane and ethyl acetate were purchased from Irama Canggih (Malaysia). Methanol was purchased from Kofa Chemical Works (Malaysia). Ascorbic acid powder was purchased from R&M Marketing (United Kingdom). DPPH powder was purchased from Sigma Aldrich (United States). Folin-Ciocalteu reagent and MTT powder were purchased from Merck (Germany). Gallic acid powder was purchased from Bio Basic Inc. (Canada). Aluminium chloride, AlCl3 powder was purchased from GENE (Malaysia). Potassium acetate powder was purchased from DAEJUNG (Korea). Quercetin powder was purchased from Acros Organics (United States). Sodium carbonate, Na_2CO_3 powder was purchased from SYSTERM (Malaysia). Vinblastine sulphate was purchased from Calbiochem (United States).

Preparation of Leaf Extracts

The harvested durian leaves from Raub, Pahang (Malaysia) were washed using tap water, air-dried and grinded into slightly fine powder form. The extraction of Durio zibethinus Linn (D197) leaves was performed via plant maceration technique utilizing four different solvents of increasing polarity namely hexane, ethyl acetate, methanol and aqueous methanol [13]. The solvent-soaked plant materials were placed in an orbital shaker at 110 rpm under room temperature for three days. The extract solution was then strained off and filtered using cheese cloth and filter paper. This was aided with centrifugation at 2000 rpm for 10 minutes for the removal of fine leaves powder. The filtrates were subjected to evaporation using rotary vacuum evaporator to obtain concentrated leaf extracts [14].

Antioxidant Assay (DPPH Free Radical Scavenging Activity)

The DPPH assay was performed in a 96-well plate and carried out in a dark environment. Serial dilution was performed in order to obtain leaf extracts of various concentrations (18.75, 37.50, 75.00, 150.00, 300.00 and 600.00 µg/mL). Approximately 100 µL of DPPH solution was added. The 96-well plate was subsequently wrapped with aluminium foil and incubated for 30 minutes at room temperature. The absorbance of the test samples was measured at a wavelength of 517 nm using a microplate reader at the end of the incubation period [15]. All of the above-mentioned steps were repeated for ascorbic acid. This assay was performed in triplicate. The DPPH free radical scavenging activity (%) of the test samples and ascorbic acid were calculated employing the formula below [16]:

$$1 - \left(\frac{\text{Average absorbance of sample}}{\text{Absorbance of negative control}} \right) \times 100\ \%$$

Determination of Total Phenolic Content (Folin-Ciocalteu Assay)

About 100 µL of each test sample (1 mg/mL) was mixed with 500 µL of FCR solution in a dark environment. The mixture was allowed to react for about three minutes at room temperature. Subsequently, about 400 µL of sodium carbonate solution was added into each tube and allowed to stand at room temperature for a period of two hours. At the end of the incubation period, 100 µL of each sample was transferred into a 96-well plate. The absorbance was measured at a wavelength of 765 nm using a microplate reader. The similar steps were repeated on the standard solutions (6.25, 12.50, 25.00, 50.00, 100.00 and 200.00 µg/mL) of gallic acid [17]. The total phenolic content of each Durio zibethinus Linn (D197) leaf extract was

expressed as µg gallic acid equivalent per mg of the extract (µg GAE/ mg of extract). This test was performed in triplicate.

Total Flavonoid Content (Aluminium Chloride Colourimetric Method)

About 100 µL of each stock test sample (1 mg/mL) was mixed with 300 µL of methanol. This was followed by addition of 20 µL of 10% aluminium chloride solution, 20 µL of potassium acetate solution and 560 µL of distilled water respectively into each microcentrifuge tube. The tube was shaken and allowed to stand at room temperature for a period of fifteen minutes. At the end of the incubation period, 100 µL of each sample was transferred into a 96-well plate and the absorbance was measured at a wavelength of 415 nm using a microplate reader. Similar steps were repeated on standard solutions (6.25, 12.50, 25.00, 50.00, 100.00 and 200.00 µg/mL) of quercetin [18]. The total flavonoid content of each Durio zibethinus Linn (D197) leaf extract was expressed as µg quercetin equivalent per mg of the extract (µg QE/ mg of extract). This test was performed in triplicate.

Determination of Cytotoxic Properties (MTT Assay)

The 96-well flat bottom microplate was seeded with 100 µL of cell suspension yielding cell concentration of $1x10^4$ cells/well and incubated for 24 hours. Leaf extracts treatment were performed in triplicate at varying concentrations (20, 40, 80, 160 and 320 µg/mL) by adding 100 µL of filtered leaf extracts. The plates were incubated at different incubation periods which were 24, 48 and 72 hours. For the positive control, vinblastine of five different concentrations were used and 0.64% DMSO solution was used as a negative control. At the end of the incubation period (24, 48 and 72 hours respectively), 20 µL of MTT solution was added into each well in the absence of light. The plate was covered with aluminium foil and incubated for another three hours at 37°C in 5% CO_2 humidified incubator. The solution in the wells were later carefully aspirated out and 200 µL of DMSO was added right after [19]. The plate was then agitated for about 5 minutes and the absorbance was read under wavelength of 570 nm. The formula for calculating the cell viability percentage was as follow [20]:

$$\text{Percentage of cell viability} = \left(\frac{\text{Average absorbance of sample}}{\text{Absorbance of negative control}}\right) \times 100\ \%$$

RESULTS

Extraction Yield of *Durio zibethinus* Linn (D197) leaf

Durio zibethinus Linn (D197) leaves were subjected to extraction with solvents of varying polarity such as hexane, ethyl acetate, methanol and aqueous methanol. The effectiveness of these solvents in active compounds extraction were manifested through their percentage of extraction yield (w/w) which was calculated based on the weight of crude extract against the weight of plant powder. Based on Fig. 1, methanol demonstrated the highest percentage of extraction yield (2.73%) among the four solvents and was followed by hexane (1.84%), ethyl acetate (1.79%) and aqueous methanol (1.71%). The extraction yields demonstrated by hexane, ethyl acetate and aqueous methanol are observed to be comparable and thus, the active compounds of the leaf are said to be equally extracted by the three solvents.

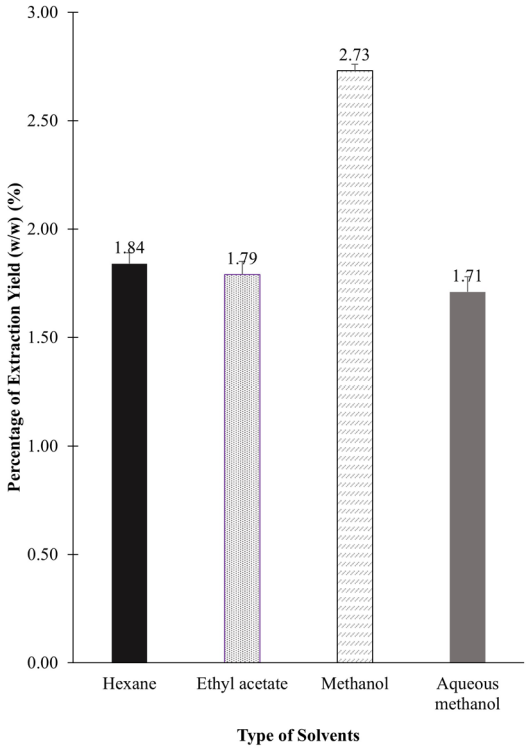

Figure 1 : Percentage of yield extracted by hexane, ethyl acetate, methanol and aqueous methanol in Durio zibethinus Linn (D197) leaf. The effectiveness of these solvents in active compounds extraction were manifested through their percentage of extraction yield (w/w) which was calculated based on the weight of crude extract against the weight of plant powder. Highest percentage of extraction yield was contributed by methanolic leaf extract. Data are given as means ± SD (n = 3).

In vitro Antioxidant Assay (DPPH Free Radical Scavenging Activity)

The antioxidant activity of Durio zibethinus Linn (D197) leaf extracts were evaluated via DPPH free radical scavenging activity using series of concentrations (18.75 µg/mL to 600.00 µg/mL) of hexane, ethyl acetate, methanol and aqueous methanol leaf extracts. Ascorbic acid was utilized as the positive control accounting to its superior radical scavenging property. Based on Fig. 2, a dose dependent relationship was demonstrated in all the four leaf extracts. This is exhibited through the increase in free

radical scavenging activity with the increase in leaf extracts concentration.

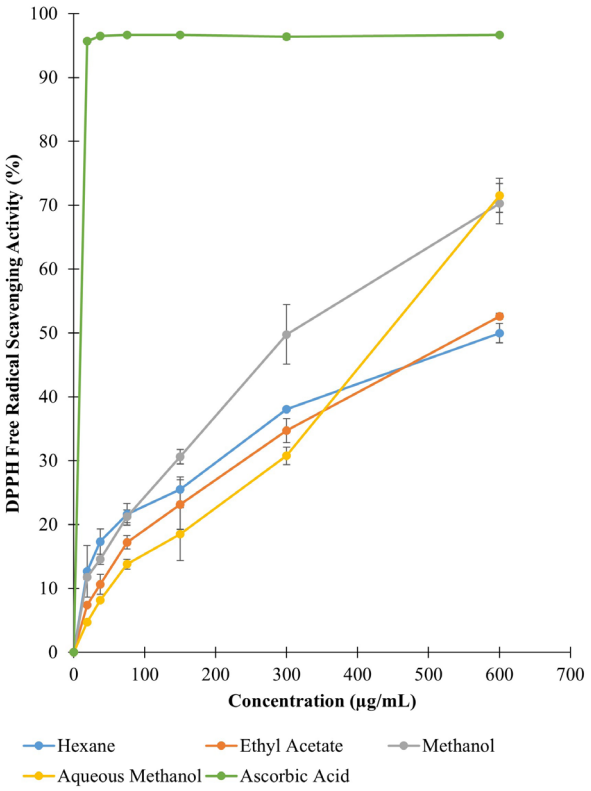

Figure 2 : Percentage of DPPH free radical scavenging activity of ascorbic acid and leaf extracts of Durio zibethinus Linn D197 (hexane, ethyl acetate, methanol and aqueous methanol). Serial dilution was performed on the substock solution of test samples in order to obtain test samples of various concentrations (18.75, 37.50, 75.00, 150.00, 300.00 and 600.00 µg/mL). Data are given as means ± SD (n = 3).

Based on Table I, the EC50 value of the four leaf extracts increased following the order of : methanol < aqueous methanol < ethyl acetate < hexane. Among the four leaf extracts, methanol conferred the highest potency as smaller concentration (304.29 µg/mL) was needed to scavenge 50% of DPPH free radicals in comparison to other extracts. This was followed by lower potency of aqueous methanol and ethyl acetate with EC50 value of 441.25 µg/mL and 556.71 µg/mL respectively. Hexane leaf extract was the least potent with more than 600 µg/mL of leaf extracts required to achieve 50% free radicals scavenging activity in DPPH. However, as a whole, ascorbic acid was the most potent free radical scavenger as compared to the leaf extracts with demonstrated ability to scavenge 50% of DPPH free radicals at a minute concentration of 9.80 µg/mL.
[Table I]

Total Phenolic Content of Durio zibethinus Linn (D197) leaf extracts

The leaf extracts of Durio zibethinus Linn (D197)

Table I : EC_{50} value of all four *Durio zibethinus* Linn D197 leaf extracts (hexane, ethyl acetate, methanol and aqueous methanol) and ascorbic acid in DPPH free radical scavenging assay.

Extract	EC_{50} Value (µg/mL)
Hexane	> 600
Ethyl Acetate	556.71
Methanol	304.29
Aqueous Methanol	441.25
Ascorbic Acid	9.80

namely hexane, ethyl acetate, methanol and aqueous methanol extracts were quantified for their total phenolic content via Folin-Ciocalteu assay. Absorbance of the extracts at 765 nm were compared to gallic acid standard curve of various concentrations (6.25 µg/mL to 200.00 µg/mL). The total phenolic content was subsequently calculated by utilizing the mathematical equation obtained from the standard curve in Fig. 3.

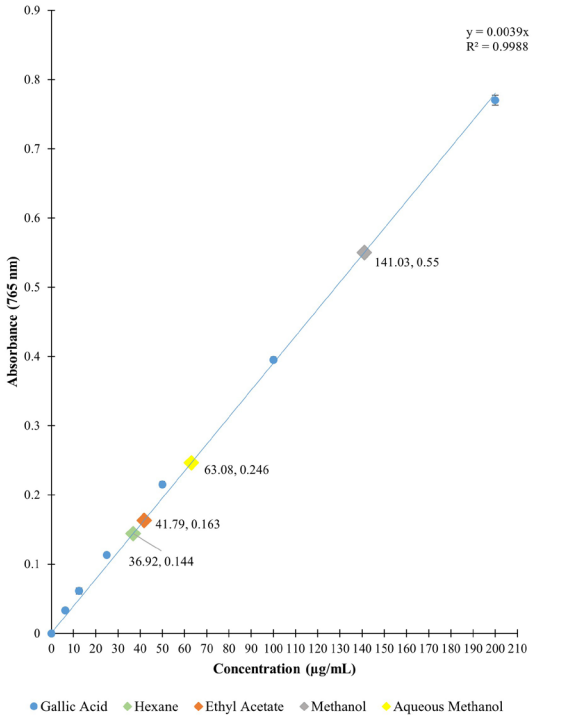

Figure 3 : Standard curve of different concentrations of gallic acid in the leaf extracts of Durio zibethinus Linn D197 (hexane, ethyl acetate, methanol and aqueous methanol). The marked diamond shaped points on the standard curve represent the relative concentration of gallic acid present in 1 mg/mL of Durio zibethinus Linn leaf extracts in correspond to their absorbance value. Methanol extract exhibited the highest total phenolic content followed by aqueous methanol, ethyl acetate and hexane extracts. Data are given as means ± SD (n = 3).

The absorbance of the gallic acid was observed to be directly proportional to its concentration. As shown in Figure 3, the total phenolic content of the four leaf extracts decreased following the decreased value of absorbance in the order of :methanol > aqueous methanol > ethyl acetate > hexane. Methanol was demonstrated to possess ability in extracting phenolic compounds (141.03 µg GAE/mg) more readily in Durio zibethinus Linn (D197) leaf as compared to aqueous methanol (63.08 µg GAE/mg), ethyl acetate (41.79 µg GAE/mg) and hexane (36.92 µg GAE/mg). The total phenolic content was expressed as gallic acid equivalent concentration unit (µg GAE/mg).

Total Flavonoid Content of Durio zibethinus Linn (D197) leaf extracts

Aluminium chloride colourimetric method was utilized for the quantification of total flavonoid content in leaf extracts of Durio zibethinus Linn (D197). Absorbance of the extracts at 415 nm were compared to quercetin standard curve of series of concentrations (6.25 µg/mL to 200.00 µg/mL). The total flavonoid content was subsequently calculated by employing the mathematical equation obtained from the standard curve in Fig. 4. The absorbance of quercetin was observed to be directly proportional to its concentration. As shown in Fig. 4, the total flavonoid

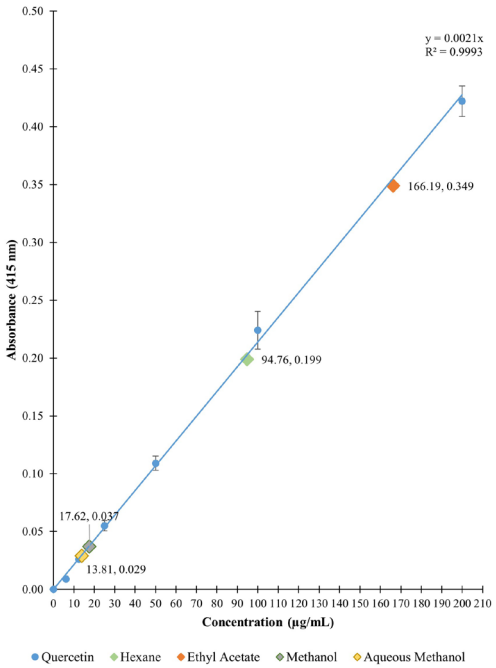

Figure 4 : Standard curve of different concentrations of quercetin acid in the leaf extracts of Durio zibethinus Linn D197 (hexane, ethyl acetate, methanol and aqueous methanol). The marked diamond shaped points on the standard curve represent the relative concentration of quercetin present in 1 mg/mL of Durio zibethinus Linn leaf extracts in correspond to their absorbance value. Total flavonoid content was the highest in ethyl acetate followed by hexane and methanol extracts. Data are given as means ± SD (n = 3).

content of the four leaf extracts declined following the order of :ethyl acetate > hexane > methanol > aqueous methanol. Ethyl acetate was found to be the solvent that possessed highest effectiveness in flavonoids extraction (166.19 µg QE/mg) of Durio zibethinus Linn (D197) leaf as compared to hexane (94.76 µg QE/mg), methanol (17.62 µg QE/mg) and aqueous methanol (13.81 µg QE/mg). The total flavonoid content was expressed as quercetin equivalent concentration unit (µg QE/mg).

In vitro Cytotoxicity Testing

The percentage of cell viability was evaluated using MTT assay after three treatment periods of 24, 48 and 72 hours to observe the effect of incubation period on cell viability. Different concentrations of leaf extracts and vinblastine (20 µg/mL to 320 µg/mL) were also utilized for assessment on the dose-response correspondence. Vinblastine which is an anti-cancer drug was included in this assay and served the purpose of positive control. As shown in Table II, the IC50 value of the four leaf extracts for treatment period of 24 hours increased following the order of :ethyl acetate < aqueous methanol < hexane < methanol. For treatment period of 48 hours, the IC50 value of the four leaf extracts increased following the order of :ethyl acetate < methanol < hexane < aqueous methanol. As for the treatment period of 72 hours, the IC50 value of the four leaf extracts increased following the order of :ethyl acetate < hexane < methanol < aqueous methanol.

Comparing the IC50 values of the four different extracts, ethyl acetate emerged as the most potent extract in inhibiting HeLa cells accounting to its lowest IC50 values of 19.95 µg/mL, 30.07 µg/mL and 23.42 µg/mL across the three treatment periods respectively. As for hexane, methanol and aqueous methanol, their cytotoxic potency on HeLa cells varied and interchanged among each other across the three period of incubations. However, aqueous methanol was observed to be the least potent extract in inhibiting HeLa cells which was manifested through its highest IC50 values (143.70 µg/mL and 143.75 µg/mL) in two incubation periods of 48 and 72 hours respectively. Vinblastine demonstrated high cytotoxic effect on HeLa cells as very minute concentration was sufficient to kill 50% of the cervical cancer cells in vitro for the three incubation periods.

Based on Fig. 5, comparison of the IC50 values across the three incubation periods showed that only vinblastine was able to exert cytotoxic effect on HeLa cells in a time dependent manner with observable decrease in cell viability across the three incubation periods. Methanolic leaf extract also possessed capability in demonstrating certain extent of time dependent inhibition with less observable decrease

Table II : IC_{50} value of all four leaf extracts of Durio zibethinus Linn D197 (hexane, ethyl acetate, methanol and aqueous methanol) and vinblastine in MTT assay after 24, 48 and 72 hours of treatment period.

Treatment	IC_{50} Value (µg/mL)		
	24 hours	48 hours	72 hours
Hexane	44.93	73.84	48.22
Ethyl acetate	19.95	30.07	23.42
Methanol	68.06	70.65	66.71
Aqueous methanol	34.44	143.70	143.75
Vinblastine	31.98	16.17	14.66

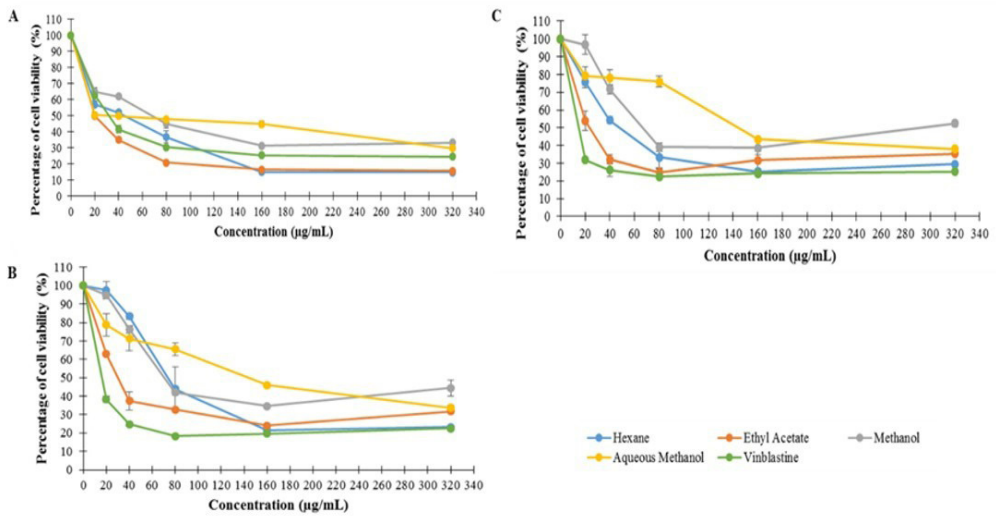

Figure 5 : Percentage of HeLa cell viability against different concentration of Durio zibethinus Linn leaf extracts (hexane, ethyl acetate, methanol and aqueous methanol) and vinblastine after (A) 24 hours, (B) 48 hours and (C) 72 hours of treatment period. Ethyl acetate emerged as the most potent leaf extracts inhibiting HeLa cells as it possessed the lowest IC50 values across the 3 incubation periods when compared to the other extracts Data are given as means ± SD (n = 3).

in the cell viability across the three incubation periods. For the effect of extract concentration, a dose dependent relationship was observed in aqueous methanol leaf extract across the three incubation periods. Hexane, ethyl acetate and methanol were only able to inhibit HeLa cells in a complete dose dependent manner in 24 hours treatment period. However, in 48 hours and 72 hours treatment period, the dose dependent curves were only valid until certain concentrations.

DISCUSSION

The choice of solvent system dictates the nature of bioactive compounds being recovered from plant materials [17]. Based on Fig. 1, the highest percentage of extraction yield was contributed by methanolic leaf extract. Hexane, ethyl acetate and aqueous methanol demonstrated comparably similar percentage of extraction yields which revealed that active compounds in the leaf were almost equally extracted by the three solvents. According to Sasidharan et al. (2011), polar solvents such as methanol, ethyl acetate and water are particularly effective for the extraction of hydrophilic compounds while non-polar solvents such as hexane is commonly utilized for extraction of lipophilic compounds [7]. Thus, methanol which emerged as the most effective solvent in this study serves as an indication that significant phytochemicals in the leaf of Durio zibethinus Linn (D197) are of polar nature. Tannins and saponins may possibly be among the polar bioactive compounds extracted as a study performed by Brown (1997) revealed detectable level of both the hydrophilic compounds in durian leaf

extract [22,23]. Due to the limited study performed on the leaf of Durio genus, comparison with previous extraction yields using various solvents was unable to be conducted.

Based on Table I, methanol leaf extract possessed the most potency in free radical scavenging followed by leaf extracts of aqueous methanol, ethyl acetate and hexane. The EC50 values of the four extracts revealed that the free radical scavenging activity followed the polarity of the solvents. As antioxidants potential in natural products is associated to the presence of phenolic compounds, the greater solubility of polyphenolic compounds in polar solvents may explain the relatively higher antioxidant activity of Durio zibethinus Linn (D197) methanolic leaf extracts as compared to the less polar extracts [24,25]. In the context of DPPH assay, it is possible to say that polar solvents notably methanol possessed greater ability in extracting phenolic compounds of ideal structure for free radicals scavenging in which the presence of hydroxyls act as hydrogen donors [26].

Due to the lack of study performed on the leaf of Durio genus, comparison with the antioxidant activity of its fruit pulp using similar solvents is made instead. An antioxidant study performed by Poovarodom et al. (2010) using different fruit pulp extracts of Durio zibethinus showed that water extract had significantly higher antioxidant activity (10.72 ± 0.5 μM TE g-1 DW) in DPPH assay as compared to methanol (6.39 ± 0.3 μM TE g-1 DW) and hexane (2.01 ± 0.1 μM TE g-1 DW) extracts [27]. This study is in agreement with the higher antioxidant activity demonstrated by more polar extracts such as methanol and aqueous methanol leaf extracts of Durio zibethinus Linn (D197).

The reported appreciable level of tannins in durian leaf extract by Brown (1997) may be among the phenolic compounds that contributed to the antioxidant activity of Durio zibethinus Linn (D197) from polar leaf extracts [22]. The roles of tannins as antioxidants are manifested via their ability to donate hydrogen atom or electrons and also chelate metal ions [28]. Apart from tannins, other phenolic compounds such as flavonoids, anthocyanins and carotenoids which were reported in durian flesh may also present in the leaves and subsequently may contribute to the antioxidant activity of Durio zibethinus Linn (D197) leaf extracts as well.

Based on Fig. 3, methanol exhibited the highest total phenolic content followed by aqueous methanol, ethyl acetate and hexane. The phenolic contents of the four extracts revealed that their total phenolic contents followed the extract polarity whereby higher total phenolics content was observed in polar solvents as compared to the non-polar ones. Due to the limited study performed on the leaf of Durio genus, comparison with the total phenolic content of its fruit pulp utilizing similar solvents is performed instead. The results obtained in this study was in agreement with a study performed by Poovadorom et al. (2010) whereby the total polyphenols quantified from the fruit pulp of Durio zibethinus was also the highest in methanol (3.65 ± 0.2 mg GAE/g) followed by water (2.61 ± 0.1 mg GAE/g) and hexane (0.47 ± 0.02 mg GAE/g) [27]. Another study performed by Haruenkit et al., (2010) also revealed higher level of polyphenols (12.3 mg GAE/g) in methanolic pulp extract of Mon Thong durian cultivar in comparison to its water extract (10.3 mg GAE/g) [29].

Phenolic compound that was reported in the leaves of Durio genus includes tannins [22]. Apart from that, phenolic compounds that were present in the pulp of Durio zibethinus such as flavonoids and carotenoids are also speculated to may have present in the leaves as well [30]. In this study, antioxidant activity and total phenolic content of leaf extracts of Durio zibethinus Linn (D197) followed the same sequence of solvents which were methanol > aqueous methanol > ethyl acetate > hexane. High polar solvents such as methanol and aqueous methanol yielded higher amount of total phenolic content and antioxidant activity.

Based on Fig. 4, the total flavonoid content of the four leaf extracts was the highest in ethyl acetate followed by hexane and methanol. Aqueous methanol possessed the lowest flavonoids content in its leaf extract. In this study, flavonoid compounds showed preference to semi-polar solvent which is ethyl acetate and non-polar solvent as compared to highly polar solvents like methanol and aqueous methanol. This may serve as an indication that the dominant flavonoid compounds in the leaves of Durio zibethinus Linn (D197) are of non-polar properties. Accounting to the limited study performed on the leaf of Durio genus, comparison with the total flavonoids content of its fruit pulp utilizing similar solvents is performed instead. A study performed by Poovarodom et al., (2010) revealed that the flavonoids content in fruit pulp of Durio zibethinus was higher in methanol extract (2.571 ± 0.1 mg CE/g) as compared to hexane (0.730 ± 0.03 mg CE/g) [27]. In a study performed by Ansari (2016), the main flavonoids found in durian flesh are quercetin and caffeic acid [31]. Thus, it is speculated that these two flavonoids may make up a part of the total flavonoids content of Durio zibethinus Linn (D197) leaf extracts.

In the evaluation of cytotoxic potential of the leaf extracts, ethyl acetate emerged as the most potent leaf extracts inhibiting HeLa cells as it possessed the lowest IC50 values across the three incubation periods when compared to the three other extracts in

Table II. On the other hand, the highest IC50 value of aqueous methanol extract in 48 and 72 hours causes it to be the least potent in exerting cytotoxic effect on HeLa cells. In this study, the cytotoxic strength and the total flavonoids content of the leaf of Durio zibethinus Linn (D197) was revealed to be in correspondence whereby the greater cytotoxic effect inflicted on Hela cells was contributed by extracts that yielded greater flavonoids content in the aluminium chloride colourimetric method. Thus, it is proposed that flavonoids could be responsible for the cytotoxic effect on HeLa cells in Durio zibethinus Linn (D197) leaf extracts.

One of the reported anticarcinogenic mechanism by flavonoids is the easy binding and penetration of flavonoids to the cell membrane of in vitro cultured cells which permits modulation of cellular metabolic activities of the tumour cells [32]. Besides that, the capability of flavonoids to modulate numerous cell cycle regulatory proteins are also discovered to result in arrestment of cell cycle progression particularly at either G1/S or G2/M phase [33,34,35]. Flavonoids also play a part in terminating the enhancement of phospholipid metabolism by tumour promoters [36]. Due to lack of cytotoxicity study performed on the Durio genus, comparison with previous cytotoxic effect of Durio leaf extracts on HeLa cell line was unable to be conducted. However, a study performed on the fruit pulp of Durio kutejensis which is under the same genus as Durio zibethinus demonstrated higher potency of ethyl acetate (47% inhibition) extract in inhibiting B16 melanoma cells as compared to very polar and non-polar solvents [37].

The phytochemical source contributing to HeLa cytotoxicity extracted from ethyl acetate was not well studied and thus, further speculation of its content is necessary. A study performed by Ansari (2016) revealed quercetin as the main flavonoids found in the durian flesh [31]. Thus, it is speculated that one of the possible flavonoids which contributed to the high cytotoxicity activity of ethyl acetate leaf extracts may be quercetin. Quercetin is able to arrest cell cycle progression at the G_1/S phase or the G_2/M transitional boundary [38]. In the context of HeLa cells, quercetin is reported to be able to inhibit the enhancement of [32P] inorganic phosphate incorporation into the phospholipid of HeLa cells by 12-O-tetracanoylphorbol-13-acetate (TPA) which is a potent tumour promoter [36].

Apart from flavonoids, the cytotoxicity effect of Durio zibethinus Linn (D197) leaf extracts against HeLa cells may also be attributed to the presence of active compounds such as tannins and saponins. This is because detectable levels of tannins and saponins have been reported by Brown (1997) in durian leaf extracts [22]. Tannins and saponins are both involved in the modulation of carcinogenesis as they are able to induce mechanisms against the progression of tumour formation. For instance, tannins possess capability to induce caspase-3-dependent apoptosis in cancer cell lines which was accounted to mechanisms such as cell cycle arrest, extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) pathway blockage. Apart from that, tannins are also able to inhibit transcription factors activation such as protein kinase C, activator protein-1 (AP1) and growth factor mediated pathways suppression [39].

The cytotoxic mechanisms of saponins involve apoptosis inducement or non-apoptotic cell death stimulation in the tumour cells [40]. According to Sak (2014), other potent cytotoxic agents for cervical cancer which are of flavonoids origin include apigenin, chrysin and luteolin [41]. A study performed by Poovarodom et al. (2010) revealed the detection of apigenin in durian pulp in which the presence of this compound in the durian leaves may be of great possibility as well [32].

A dose dependent relationship was exhibited in aqueous methanol leaf extract across the three incubation periods. As for hexane, methanol, ethyl acetate and vinblastine, the cytotoxic effect on HeLa cells were only exhibited in a complete dose dependent manner in 24 hours incubation period. Towards the concentration of 320 µg/mL of the leaf extracts except for aqueous methanol extract, decreased inhibition rate on HeLa cells was observed. This effect is speculated to be due to the induction of resistance of the cells at higher drug concentration [43]. Comparing the cytotoxic trends of vinblastine and the leaf extracts, the leaf extracts particularly ethyl acetate leaf extract exerted its action in a short-acting drug manner in which it was most potent in 24 hours of treatment period. As for vinblastine, its most potent cytotoxicity exhibited in later hours of incubation (48 and 72 hours) may indicate the exertion of its action in a long-acting manner.

Further studies are essential for the exploration of bioactive compounds present in the leaf extracts of Durio zibethinus Linn (D197). This is to allow revelation of bioactive compounds extracted by each solvent that have contributed to the antioxidant and cytotoxic activities of the leaf extracts. Several techniques that can be employed for this isolation or purification purpose include high performance liquid chromatography (HPLC), thin layer chromatography (TLC) and column chromatography [43]. Apart from that, the cytotoxic properties of the leaf extracts can also be evaluated in other types of cancer cell line. This is because the anticancer effect exhibited by each solvent can vary in different cancer cell lines. Normal cell lines can also be utilized as part of the

study for evaluation on the specificity of the bioactive compounds. This is to ensure that the extracted compounds are only toxic to malignant cells and not normal cells.

CONCLUSION

This study demonstrated methanol as high polar s olvent yielded a higher amount of total phenolic content and antioxidant activity. The greatest cytotoxicity exhibited by ethyl acetate leaf extract on HeLa cells may be attributed to its highest flavonoid content. Holistically, the potential of Durio zibethinus Linn (D197) leaf extracts as natural antioxidants which can serve as an alternative to the utilization of synthetic antioxidants apart from its possible contribution to cancer treatments were deemed conceivable. Having said of that, future studies concerning the isolation and identification of the extracted active compounds will certainly aid in the validation of the information obtained in this study.

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