Q-TOF LC-MS compounds evaluation of propolis extract derived from Malaysian stingless bees, *Tetrigona apicalis*, and their bioactivities in breast cancer cell, MCF7

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A B S T R A C T

Propolis is known to exhibit various phytochemical compounds that aid in several biological activities. The current study investigates the phytochemical compounds of ethanolic extract of propolis of *Tetrigona apicalis* (EEP) using Q-TOF LC-MS, its antioxidant properties using DPPH and ABTS* radical scavenging assays, total phenolic (TPC) and flavonoid content (TFC), using Folin-Ciocalteu and Aluminium Chloride method, respectively, as well as proapoptotic effects, based on the selected IC50 of the cytotoxic study conducted for EEP using annexin V-FITC assay. Terpene and polyphenol were among of 17 identified compounds. The EC50 of EEP for DPPH and ABTS* was 1.78 mg/mL and 1.68 mg/mL, while the EEP exhibited TPC and TFC values of 31.99 mgGAE/g and 66.4 mgQCE/g, respectively in which the parameters were strongly correlated. The IC50 of EEP effectively induces apoptosis in MCF7 cells. In conclusion, EEP possessed important phytochemical compounds that work excellently as antioxidants and anticancer agents.

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1. Introduction

Propolis has long been used as a part of herbal medicine in several countries. This traditional herbal medicine is made from sticky, resinous materials from various plant sources that served as a "glue" to repair any cracks or holes in the bee’s nest. Propolis, which is derived from the Greek word, “pro-” (in defense or bar-
on the anticancer activities of Malaysian propolis are still scarce, with only a few cell lines were investigated such as SK-UT-1 (uterine leiomyosarcoma cells), Hela (cervical cancer cells), and MDA-MB-231 (breast cancer cells) (Gapar 2018; Mat Nafi et al. 2019). Nevertheless, the other specific biological benefits of each stingless bee species have yet to be explored, as there are 17 to 32 well-known species of stingless bees in Malaysia (Kelly et al. 2014).

The current study is a continuation of a previous study on Tetragona apicalis propolis extract conducted by Mohamed et al. (2020). T. apicalis was discovered in Southeast Asia, Indo-Malaya/Australasia, and particularly in the subtropical regions (Rasmussen 2008). This species, as one of the three most common species of Malaysian stingless bees, is native to the wild, as opposed to the other two species, Heterotrigona itama, and Geniotrigona thoracica, which are kept for beekeeping/meliponiculture (Kelly et al. 2014). This characteristic has resulted in T. apicalis being a potent pollinator group in most ecosystems, especially in Malaysian virgin jungle reserves (Jauker et al. 2012; Salim et al. 2012).

Up to this point, there have been no studies that have focused on the screening of bioactive compounds using Q-TOF LC-MS with the proapoptotic potential of T. apicalis propolis extract towards hormone-responsive breast cancer, MCF7. Thus, this study aims to identify potential phytochemical compounds in T. apicalis propolis extract using Q-TOF LC-MS and evaluates its antioxidant properties, along with total phenolic and flavonoid content, in which these components may contribute to anticancer activities. Additionally, the present study also uses IC50 values from cytotoxic activity as described by Mohamed et al. (2020) to conduct apoptosis induction assay. The findings of the current study will hypothetically demonstrate the potential of T. apicalis propolis extract to be a potent anticancer agent, particularly for hormone-responsive breast cancer in the near future.

2. Materials and methods

2.1. Materials

The analytical grade (dimethyl sulfoxide (DMSO), ethanol, and methanol), powder form of 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS’), potassium persulphate (K2S2O8), sodium carbonate (Na2CO3), sodium nitrite (NaNO2), aluminum chloride (AlCl3), sodium hydroxide (NaOH), Folin-Ciocalteu reagent, standards for cytotoxic assay (tamoxifen), standard for antioxidant assays (quercetin, hydroxide (NaOH)), Folin-Ciocalteu reagent, standards for cytotoxic assay (tamoxifen), standard for antioxidant assays (quercetin, hydroxide (NaOH)), Folin-Ciocalteu reagent, standards for cytotoxic assay (tamoxifen), standard for antioxidant assays (quercetin, hydroxide (NaOH)), Folin-Ciocalteu reagent, standards for cytotoxic assay (tamoxifen), standard for antioxidant assays (quercetin, hydroxide (NaOH)), Folin-Ciocalteu reagent, standards for cytotoxic assay (tamoxifen), standard for antioxidant assays (quercetin, hydroxide (NaOH)), Folin-Ciocalteu 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Then, 150 µL of DPPH working solutions were mixed with 7.5 µL of samples in a 96-well plate and placed in the dark for 30 min at room temperature, whereas methanol was used as blank. All concentrations were measured in triplicate. The positive control (Trolox) was treated in the same conditions as the samples. The scavenging effect percentage was determined by using the equation below:

\[
\text{Inhibition(\%)} = \left( \frac{A_1 - A_2}{A_1} \right) \times 100\% 
\]

(1)

where \( A_1 \) is the absorbance of the control and \( A_2 \) is the absorbance of the samples. The mean half-maximal response of EEP concentration (EC50) value was estimated as mean ± standard deviation (SD). The EC50 for DPPH for both EEP and Trolox was calculated using four-parameter logistic regression equation calculator by AAT Bioquest (Sunnyvale, USA) (Costales-Carrera et al. 2019).

2.6. ABTS⁺ radical scavenging assay

The ABTS⁺ radical scavenging activity in EEP was evaluated using the method explained by Vongsak et al. (2015) and Campos et al. (2015) with slight modifications. About 7 mM ABTS aqueous solution and 2.45 mM potassium persulfate in water were prepared and mixed. Subsequently, the mixture was placed in the dark for 12 to 16 h at room temperature to yield a stock solution. The ABTS⁺ radical solution was prepared by reacting 1 mL ABTS⁺ radical with 50 mL methanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm using a spectrophotometer.

Samples with concentrations ranging from 0.02 to 0.313 mg/mL were prepared. About 1.25 µL of samples were allowed to mix with 125 µL of ABTS⁺ radical in a 96-well plate. The mixture was kept in the dark at 37 °C for 6 min, whereas methanol was used as blank. All concentrations were repeated in triplicates. The positive control, Trolox, was used in the same setting as the samples. The percentages of scavenging effects were calculated by the equation below:

\[
\text{Inhibition(\%)} = \left( \frac{A_1 - A_2}{A_1} \right) \times 100\% 
\]

(2)

where \( A_1 \) is the absorbance of the control and \( A_2 \) is the absorbance of the samples. The EC50 value was calculated as mean ± SD. The EC50 for ABTS⁺ was calculated using linear regression equations, in which the regression curve for TPC (with quercetin as a standard) comprises the equation \( y = 0.0157x - 0.0675 \), with \( R^2 = 0.9959 \).

2.7. Total phenolic compounds (TPC)

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method with slight modifications (Kothai and Jayanthi 2014). The samples with various concentrations ranging from 0.1 to 12.5 mg/mL were initially dissolved with 1 mL of methanol. An amount of 10 µL of each sample was then allowed to react with 3 mL and 40 µL of NaNO2 and distilled water, respectively. About 3 µL of 10 % AlCl3 was added after 5 min, followed by 20 µL of sodium hydroxide solution after the former solution was mixed had been mixed for 5 min. The absorbance was read using a spectrophotometer at 420 nm with quercetin as a standard reference. The TPC of EEP was expressed as quercetin equivalent (QCE) in µg/g dry weight of the extract using standard curve valued 6.25 to 100 µg/mL. The regression curve for TPC (with quercetin as a standard) comprises the equation \( y = 0.0157x - 0.0675 \), with \( R^2 = 0.9959 \).

2.8. Total flavonoid compounds (TFC)

The total flavonoid contents (TFC) were determined using the Aluminium Chloride Method with some modifications (Fidrianny et al. 2015). The samples with various concentrations from 0.1 to 12.5 mg/mL were initially dissolved with 1 mL of methanol. An amount of 10 µL of each sample was then allowed to react with 3 mL and 40 µL of NaNO2 and distilled water, respectively. About 3 µL of 10 % AlCl3 was added after 5 min, followed by 20 µL of sodium hydroxide solution after the former solution was mixed had been mixed for 5 min. The absorbance was read using a spectrophotometer at 420 nm with quercetin as a standard reference. The TFC of EEP was expressed as quercetin equivalent (QCE) in µg/g dry weight of the extract using standard curve valued 6.25 to 100 µg/mL. The regression curve for TFC (with quercetin as a standard) comprises the equation \( y = 0.0157x - 0.0675 \), with \( R^2 = 0.9959 \).

2.10. Statistical analysis

The statistical analysis was done in three replicates, and the data were evaluated as mean values with standard deviation (SD), with p-values < 0.05 considered significant. The percentage of apoptosis induction for selected IC50 of EEP and Tamoxifen was calculated using an independent sample t-test, whereas the relationship between antioxidant activity with TPC and TFC was calculated using Pearson’s correlation coefficient. Both calculations were done using International Business Machine Corporation Statistical Product and Service Solutions (IBM SPSS) Statistics Version 27.

3. Results

3.1. Extraction of T. Apicalis propolis extract

The yield (%) of the crude extract along with its physical appearance was deliberated and documented. The crude extract was
whitish in appearance, and the samples were in powder form. The EEP sample that was derived from crude ethanolic extract produced a yield of 57 %.

3.2. Q-TOF LC-MS analysis

The detection of phytochemical compounds in the extract using Q-TOF LC-MS was analyzed on EEP with separation of major bioactive compounds using LC and identification via MS with positive and negative mode ESI. The score of similarities was supported by the molecular feature extraction (MFE) algorithm and molecular formula generator (MFG) software. Using at least 80 % similarities with chemical compounds from the METLIN library, the results of Q-TOF LC-MS analysis are summarized in Table 1(a) and Table 1(b).

The phytochemical compounds of propolis previously identified in several studies were used as external standards in order to accentuate the findings of the extract (Midorikawa et al. 2001; Carvalho et al. 2011). A study on Malaysian *H. itama* propolis extract conducted by Zhao et al. (2017) discovered the presence of several important bioactive compounds, such as gallic acid, kaempferol, and caffeic acid. Thus, the standards as described by Zhao et al. (2017) were incorporated in the screening of the present study.

Based on Table 1(a) and Table 1(b), 17 compounds have been identified, with 12 compounds from positive ESI and 5 compounds from negative ESI. The LC-MS also detected two compounds that had the same retention time but different *m/z*. At retention time 15.720 in positive ESI, prolyl-alanyl-lysine and 1-hexanol arabinosylglucoside were identified with each fragmentation ions of 15.720 in positive ESI, prolyl-alanyl-lysine and 1-hexanol arabinosylglucoside did not exhibit any compounds that were matched with external standards. The significance of the identified compounds will be explained later in the discussion section.

3.3. Determination of DPPH and ABTS\(^{+}\) radical scavenging activity

The DPPH and ABTS\(^{+}\) radical scavenging activities of EEP were determined for several concentrations to signify the presence of potential antioxidant activities. Table 2 lists the calculated concentration values of EEP needed to scavenge DPPH and ABTS\(^{+}\) by half (EC\(_{50}\)). Because EEP and Trolox both used the same concentration range, the maximum DPPH and ABTS\(^{+}\) radical scavenging activities of EEP were at 0.313 mg/mL with 3.59 % and 9.5 % inhibition in correlation to 92.5 % Trolox and 49.8 % Trolox, respectively. On contrary, the lowest EEP radical scavenging activity was at 0.02 mg/mL with no inhibition and 1.2 % inhibition corresponding to 27.5 % Trolox and 7.8 % Trolox, for DPPH and ABTS\(^{+}\), respectively. The EC\(_{50}\) of EEP for DPPH and ABTS\(^{+}\) were 1.78 mg/mL and 1.68 mg/mL, respectively, whereas the EC\(_{50}\) of Trolox for DPPH and ABTS\(^{+}\) were 0.04 mg/mL and 0.31 mg/mL, respectively.

3.4. Determination of TPC and TFC

The phenolic and flavonoid contents are noted to play a significant role for antioxidant activities, particularly in propolis (Miguel et al. 2010). By using the same concentration for both tests, Table 3 shows the highest concentration of EEP (12.5 mg/mL) to exhibit total phenolic and flavonoid contents, with TPC valued at 31.99 mgGAE/g and TFC valued at 66.4 mgQCE/g.

3.5. Correlation of DPPH, ABTS\(^{+}\), TPC and TFC

The relationship between antioxidant activity with TPC and TFC was measured using the Pearson correlation coefficient, in which the correlation coefficient ranged from +1 to −1. Based on Table 4, there is a strong positive relationship between antioxidant activity with TPC and TFC, with all correlations falling between r = 0.950 and r = 0.971.

| Table 1a |
|-----------|
| Phytochemical compounds identified in EEP using Q-TOF LC-MS (Positive ESI). |
| Peak | Rt | *m/z* | Error (ppm) | Formula | MW (g/mol) | Identification | Score (MFE) | Score (MFG) |
|-------|-----|-------|-------------|---------|------------|---------------|-------------|-------------|
| 1     | 16.359 | 205.1952 | 0.47 | C\(_{18}\) H\(_{24}\) O | 204.1879 | 200.1831 | (5)-beta-himachalene Ishwarol | 203.1849 | 100 | 83.77 |
| 2     | 11.095 | 221.1904 | 1.84 | C\(_{15}\) H\(_{22}\) O | 195.1408 | 203.1824, 209.1542 | Leucyl-phenylalanine | 279.1661 | 100 | 91.44 |
| 3     | 16.708 | 279.1692 | 2.79 | C\(_{15}\) H\(_{22}\) N\(_{2}\) O\(_{3}\) | 278.1623 | 276.221 | 1-Hexanol arabinosylglucoside | 279.1661 | 100 | 80.2 |
| 4     | 16.723 | 299.2102 | 0.90 | C\(_{17}\) H\(_{22}\) N\(_{2}\) O | 276.221 | 279.1661 | Leucyl-phenylalanine | 279.1661 | 100 | 91.37 |
| 5     | 15.750 | 314.1966 | 1.90 | C\(_{15}\) H\(_{24}\) N\(_{2}\) O\(_{4}\) | 314.1966 | 314.1966 | Prolyl-alanyl-lysine | 392.2495 | 100 | 91.19 |
| 6     | 15.750 | 392.1697 | 0.34 | C\(_{17}\) H\(_{22}\) O\(_{3}\) | 392.1697 | 392.1697 | 1-Hexanol arabinosylglucoside | 392.2495 | 100 | 91.19 |
| 7     | 19.002 | 427.3666 | 3.30 | C\(_{21}\) H\(_{30}\) N\(_{2}\) O\(_{3}\) | 426.3583 | 426.3583 | 392.2495 | 100 | 91.19 |
| 8     | 16.778 | 469.3316 | 0.99 | C\(_{24}\) H\(_{44}\) N\(_{2}\) O\(_{3}\) | 468.3235 | 468.3235 | 426.3583 | 100 | 91.19 |
| 9     | 22.176 | 493.339 | 1.66 | C\(_{21}\) H\(_{30}\) N\(_{2}\) O\(_{3}\) | 470.3501 | 470.3501 | 392.2495 | 100 | 91.19 |
| 10    | 17.434 | 497.3722 | 1.86 | C\(_{21}\) H\(_{30}\) N\(_{2}\) O\(_{3}\) | 474.384 | 474.384 | 392.2495 | 100 | 91.19 |
| 11    | 15.025 | 531.3682 | 2.08 | C\(_{21}\) H\(_{30}\) N\(_{2}\) O\(_{3}\) | 530.3618 | 530.3618 | 426.3583 | 100 | 91.19 |
| 12    | 15.719 | 771.4169 | 4.46 | C\(_{30}\) H\(_{62}\) O\(_{15}\) | 770.4123 | 770.4123 | 771.4169 | 100 | 91.19 |

Note: RT, retention time; MW, molecular weight; MFG, molecular formula generator; MFE, molecular feature extraction.
Table 1b
Phytochemical compounds identified in EEP using Q-TOF LC-MS (Negative ESI).

| Peak | Rt     | m/z       | Error (ppm) | Formula       | MW (g/mol) | Identification                  | MS/MS (m/z) | Score (MFE) | Score (MFG) |
|------|--------|-----------|-------------|---------------|------------|----------------------------------|-------------|-------------|-------------|
| 1    | 14.311 | 331.1977  | 3.15        | C_{41}H_{26}N_{4}O_{3} | 332.2049   | Valine-serine-lysin              | 331.1975    | 100         | 92.41       |
| 2    | 15.717 | 419.2137  | 0.91        | C_{28}H_{30}F_{2}N_{2}O_{4} | 420.2209   | Levocabastine                    | 409.1841    | 100         | 98.21       |
| 3    | 9.612  | 515.1704  | 1.02        | C_{16}H_{32}O_{2} | 516.1779   | Rottlerin                        | 491.9059, 505.1307 | 100 | 98.45       |
| 4    | 16.722 | 631.3827  | 4.63        | C_{26}H_{30}O_{3} | 632.3895   | Oleandin acid 3-O-beta-o-         | 631.3918    | 100         | 85.4        |
| 5    | 15.718 | 747.4208  | -1.14       | C_{16}H_{32}N_{4}O_{6} | 374.217    | Leucine-aspargate-lysin          | 373.2126, 419.2169, 567.3460, 641.3202 | 100 | 95.8        |

Note: RT, retention time; MW, molecular weight; MFG, molecular formula generator; MFE, molecular feature extraction.

Table 2
The concentrations of EEP and Trolox with DPPH and ABTS + radical scavenging activity and its corresponding EC50.

| Concentration (mg/mL) | EEP Radical Scavenging Activity | Trolox Radical Scavenging Activity |
|-----------------------|---------------------------------|-----------------------------------|
|                       | DPPH | ABTS* | DPPH | ABTS* |
| 0.02                  | -    | 1.2 % | 27.5 % | 7.8 % |
| 0.313                 | 3.59 % | 9.5 % | 92.5 % | 49.8 % |
| EC50                  | 1.78 mg/mL | 1.68 mg/mL | 0.04 mg/mL | 0.31 mg/mL |

Table 3
The value of EEP concentration for with its maximal TPC and TFC. Data are mean ± SD of triplicate experiments.

| TPC (mgGAE/g) | TFC (mgQCE/g) |
|---------------|---------------|
| Linear Regression Equation | y = 0.0022x + 0.0103 |
| R² value | 0.9902 |
| EEP | 31.99 ± 0.01 |
| | 66.40 ± 0.01 |

Table 4
The Pearson’s correlation coefficient (r) of DPPH, ABTS*, TPC and TFC. The statistical difference was calculated using Student’s paired t-test. All are significant with p value < 0.05.

| TPC          | TFC          |
|--------------|--------------|
| DPPH         | 0.950        |
| ABTS*        | 0.971        |
| Score (MFE)  | 0.961        |
| Score (MFG)  | 0.956        |

3.6. Apoptosis induction assay of EEP
The percentage of apoptotic cells was determined using a flow cytometer, in which staining (consisted of annexin V and PI) was done to MCF7-treated with EEP, MCF7-treated with tamoxifen, and untreated cells. Table 5 shows the percentage of apoptosis induction of selected IC50 for MCF7-treated with EEP (32.70 µg/mL), MCF7-treated with tamoxifen (7.85 µg/mL) and untreated cells in three incubation points, while Fig. 1 is the flow cytometry analysis of IC50 of MCF7 and tamoxifen with untreated cells in 3 different incubation period (Mohamed et al. 2020). Based on Table 5 and Fig. 1, the apoptosis induction assay validates the cytotoxic study of selected IC50 conducted by Mohamed et al. (2020), in which the cell viability of viable, early apoptosis, late apoptosis, and necrotic/dead cells corresponded to 48.39 ± 2.06 %, 14.02 ± 0.98 %, 35.25 ± 1.16 %, and 2.34 ± 0.14 %, respectively.

4. Discussions
Overall, the detected compounds in EEP for positive ESI of Q-TOF LC-MS mostly consisted of terpene groups. Based on Table 1(a), both (S)-beta-himachalene and ishwarol belong to terpene derivatives of sesquiterpene hydrocarbon and oxygenated sesquiterpenes, respectively. Both types of sesquiterpenes have been mentioned in several studies for their potential to act as potent antioxidant and anticancer agents (Khan et al. 2008; Dahham et al. 2015; Jain et al. 2016). Xia et al. (2020) also reported that ganoderic acid DM (which belongs to triterpenoid) proves to induce autophagy apoptosis in non-small cell lung carcinoma via inhibition of PI3K/Akt/mTOR pathway. Aside from that, Tyler et al. (2015) discovered that 3-alpha-threo-1-phenyl-2-palmitoyla mino-3-morpholino-1-propanol (DL-PPMP) potentiates cisplatin cytotoxicity in acquired cisplatin-resistance of lung carcinoma and malignant pleural mesothelioma.

In terms of negative mode ESI of Q-TOF LC-MS, however, only one triterpenoid was present, which was oleanolic acid 3-O-beta-o-glucosiduronic acid. The report is similar to those of Saleem et al. (2020), as this compound was present in the methanolic extract of Bougainvillea glabra flowers. However, the compound’s ability to act as an antioxidant and anticancer agent had yet to be discovered. Rottlerin, a polyphenol compound, was also discovered in the present study. Chhiber et al. (2016) demonstrated rottlerin’s ability to act as an antioxidant, as it reduced NADPH oxidase activity, inhibited dysfunction of mitochondria, and maintained antioxidant condition.

To the best of our knowledge, this is the first time the bioactive compounds are discovered for Malaysian propolis. As since this study only focuses on phytochemical screening using Q-TOF LC-MS to illustrate the therapeutic significance of compounds from crude EEP, the selection and evaluation of potential compounds will need to be specified further with fractionation using polar and non-polar solvents, and isolation from the pure fraction with thin layer chromatography (TLC) and column chromatography.

In relation to antioxidant activities, based on the comparison of EC50 in Table 2, it is also concluded that both radical scavenging activities of DPPH and ABTS* corresponded with the concentration gradient (concentration-dependent effect). The current findings are also supported by multiple studies that agreed on the presence of antioxidant activities in T. apicalis propolis extract (Rosli et al. 2017; Asem et al. 2019). By using at least 80 % of compound similarities in Q-TOF LC-MS, it can be noted that both terpene and...
polyphenol contributed to the antioxidant activities in EEP. However, there is a lack of a definitive or optimized method to measure total terpene content in EEP because terpene constituents are the largest group of natural compounds (Indumathi et al. 2014). Therefore, the current study only focusing to determine the total phenolic content. In addition, as flavonoid is the largest subclass group of

### Table 5

| Incubation Point (h) | Cell Viability (%) | Early Apoptosis | Late Apoptosis | Necrotic Cells |
|----------------------|-------------------|----------------|---------------|---------------|
| MCF7 Treated with EEP |                   |                |               |               |
| 24                   | 88.20 ± 1.51**    | 4.09 ± 0.85*   | 7.26 ± 1.12** | 0.45 ± 0.45*  |
| 48                   | 87.58 ± 1.01**    | 3.90 ± 0.31**  | 7.53 ± 0.51** | 0.99 ± 0.26   |
| 72                   | 48.39 ± 2.06**    | 14.02 ± 0.98** | 35.25 ± 1.16**| 2.34 ± 0.14** |
| MCF7 Treated with Tamoxifen |          |                |               |               |
| 24                   | 81.9 ± 0.57***    | 9.93 ± 0.23**  | 6.18 ± 1.06** | 1.99 ± 0.72   |
| 48                   | 72.75 ± 0.79***   | 8.79 ± 0.25**  | 15.88 ± 0.35**| 2.58 ± 0.28** |
| 72                   | 41.67 ± 1.99***   | 10.33 ± 1.16** | 46.64 ± 1.83**| 1.36 ± 0.25   |
| Untreated Cells      |                   |                |               |               |
| 24                   | 96.65 ± 0.43      | 0.92 ± 0.21    | 0.90 ± 0.08   | 1.50 ± 0.19   |
| 48                   | 93.74 ± 0.66      | 1.79 ± 0.19    | 3.41 ± 0.52   | 1.06 ± 0.12   |
| 72                   | 92.14 ± 0.66      | 2.16 ± 0.19    | 4.68 ± 0.52   | 1.02 ± 0.12   |

Note: *, p value < 0.05; **, p value < 0.01.

### Fig. 1

Flow cytometry analysis of selected IC₅₀ of EEP, Tamoxifen with untreated cells in three incubation points. Values are presented as means ± SD of triplicate experiments. The statistical analysis was estimated using independent sample t-test for EEP and Tamoxifen in comparison to untreated cells.

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polyphenols, the measurement of total flavonoid content was also done in the current study.

Based on the TPC and TFC results in Table 3, it is noted that the value of TFC is greater than the TPC value. This finding is in agreement with several studies, which included propolis extract of Malaysian stingless bees, *T. apicalis* and *H. itama* (Rosli et al. 2017; Awang et al. 2018). According to Katsube et al. (2004) and Wu et al. (2004), the most likely reason is phenolics characterized by all compounds that contained a phenolic group (monophenol, diphenol, triphenol, or polyphenol). Due to the vast complexity of compounds in phenolics, the characterization of each compound with its structure elucidation can be difficult, especially when dealing with many herbal extracts. According to Anokwuru et al. (2011), depending on the number of phenolic groups in phenolic compounds, the response towards Folin–Ciocalteu reagent might react differently. Thus, the slightly lower value in TPC in this study did not reflect the total actual value of phenols in EEP.

Additionally, as though the findings of polyphenol using Q-TOF LC-MS of the current study was only rottlerin, the contribution by other types of polyphenols in antioxidant activities may be also contributed by the polyphenols that valued < 80 % similarities from METLIN library. Nevertheless, there is a strong relationship between antioxidant activity with TPC and TFC, with all correlations falling between r = 0.950 and r = 0.971. Table 4 shows that the correlation between antioxidant activities with TPC and TFC is all strongly positive, with r-values greater than 0.9.

Based on Table 5 and Fig. 1, the apoptosis induction assay in this study validates the cytotoxic study of selected IC50 conducted by Mohamed et al. (2020), whereby the cell viability percentage of viable cells, early apoptosis, late apoptosis, and necrotic/dead cells corresponded to 48.39 ± 2.06 %, 14.02 ± 0.98 %, 35.25 ± 1.16 %, and 2.34 ± 0.14 %, respectively. In comparison to a study by Gaper (2018) for EEP of *T. apicalis*, whereby the cells percentage of early and late apoptosis phase of HeLa cells were 6 % and 23.97 %, respectively; the results for both phases in the current study was relatively higher, in which corresponded to 14.02 ± 0.98 % and 35.25 ± 1.16 %, respectively. Therefore, it was justified that EEP is more sensitive to cause apoptosis induction in early or late apoptosis in MCF7 than in HeLa cells. Thus, it can be concluded that the antioxidant capacities produced in EEP play a part to cause apoptosis induction in cancer cells. It is previously known that the antioxidants from plant origin with/without other natural sources have been shown to cause cell death through apoptosis induction in breast, lung, liver, colorectal, and alveolar cancers, in particular (Kntaya et al. 2018; Adebayo et al. 2019).

In relation to MCF7, it was reported that ganoderic acid DM that was found in the current study could induce DNA fragmentation and reduce the mitochondrial membrane potential in MCF7 cells, as reported by Wu et al. (2012). In addition, Torricelli et al. (2008) also reported that rottlerin was able to inhibit the nuclear factor κB/Cyclin-D1 cascade in MCF7, proving its anticancer activity. The molecular analysis, including the protein pathway using western blot analysis, will be recommended for future studies to confirm and validate the proteins responsible for activation of apoptosis cascade for EEP of *T. apicalis*.

5. Conclusion

In conclusion, EEP is proved to have significant bioactive compounds that was capable in various biological activities, including antioxidant and anticancer activities. Additionally, this study deduced that the apoptosis induction based on the selective IC50 of EEP conclusively signified the cytotoxic activity of EEP. The molecular validation using western blot analysis to conform the EEP apoptotic effect as well as compound fractionation and isolation may be recommended for further EEP studies.

CRediT authorship contribution statement

Wan Ahmad Syazani Mohamed: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft, Visualization. Noor Zafira Ismail: Data curation, Software, Investigation, Resources. Mustahimah Muhamad: Data curation, Software, Investigation, Resources. Eshaifol Azam Omar: Resources, Visualization, Project administration. Nozlena Abdul Samad: Resources, Visualization, Project administration. Ooi Jer Ping: Writing – review & editing. Sharlina Mohamad: Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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