Research Article

Secondary Metabolites, Antioxidant, and Antiproliferative Activities of *Dioscorea bulbifera* Leaf Collected from Endau Rompin, Johor, Malaysia

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Breast cancer is among the most commonly diagnosed cancer and the leading cause of cancer-related death among women globally. Malaysia is a country that is rich in medicinal plant species. Hence, this research aims to explore the secondary metabolites, antioxidant, and antiproliferative activities of *Dioscorea bulbifera* leaf collected from Endau Rompin, Johor, Malaysia.

Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays, while the cytotoxicity of *D. bulbifera* on MDA-MB-231 and MCF-7 breast cancer cell lines was tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell cycle analysis and apoptosis were assessed using flow cytometry analysis. Phytochemical profiling was conducted using gas chromatography-mass spectrometry (GC-MS). Results showed that methanol extract had the highest antioxidant activity in DPPH, FRAP, and ABTS assays, followed by ethyl acetate and hexane extracts. *D. bulbifera* tested against MDA-MB-231 and MCF-7 cell lines showed a pronounced cytotoxic effect with IC₅₀ values of 8.96 μg/mL, 6.88 μg/mL, and 3.27 μg/mL in MCF-7 and 14.29 μg/mL, 11.86 μg/mL, and 7.23 μg/mL in MDA-MB-231, respectively. Cell cycle analysis also indicated that *D. bulbifera* prompted apoptosis at various stages, and a significant decrease in viable cells was detected within 24 h and substantially improved after 48 h and 72 h of treatment. Phytochemical profiling of methanol extract revealed the presence of 39 metabolites such as acetic acid, n-hexadecanoic acid, acetin, hexadecanoate, 7-tetradecenal, phytol, octadecanoic acid, cholesterol, palmitic acid, and linolenate. Hence, these findings concluded that *D. bulbifera* extract has promising anticancer and natural antioxidant agents. However, further study is needed to isolate the bioactive compounds and validate the effectiveness of this extract in the *in vivo* model.

1. Introduction

Millions of mortalities were reported globally as a result of cancer, and the numbers of new cases are expected to increase in the future [1]. Breast tumour is the second highest cancer recorded worldwide and the most common cause of cancer death among women. It has been ascertained that about 25% of all new identified cancer case is breast cancer, which accounts for 15% of deaths in women each year [2, 3]. Breast cancer is a commonly diagnosed cancer in Malaysia, having a standardized age rate of 47.4 per 100,000 [4, 5]. The National Cancer Registry of Malaysia (NCR) records that 21,773 Malaysians who are perceived with cancer and approximations show that unregistered cases
have reached about 10,000 cases every year. It is likely that by 75 years old, ¼ of Malaysians will be diagnosed with cancer [6]. Therefore, discovering new drugs with minimal toxicity is a broad scientific challenge, and there is an immediate need to search for a new agent to mitigate the menace by using alternative evidence-based herbal medicines.

*Dioscorea bulbifera* (Figure 1) is a member of the family Dioscoreaceae, categorised in the order Dioscoreales and genus Dioscorea. It is usually referred to as air potato, air yam, or bulbil-bearing yam. It is a prolific climbing plant indigenous to Southern Asia and West Africa, mostly found along forest edges. The direction of circular twinning had been reported in *D. bulbifera* and can grow to a height of 12 m. It has shiny green leaves that are alternate with a long leafstalk. Resembling true yam leaves, *D. bulbifera* has an annual vegetative cycle. Hence, this study was directed toward discovering secondary metabolites, antioxidant, and antiproliferative effects of *D. bulbifera* leaf extract from Kampung Peta, Endau Rompin, Mersing, Johor, Malaysia.

**2. Materials and Methods**

2.1. Sample Collection and Preparation. *D. bulbifera* fresh leaves were collected at Kampung Peta, Johor, Malaysia, on 5th May 2017 with the permission of Perbadanan Taman Negara Johor (PTJN) and with full observation of rules and regulations on collection practices of medicinal plants as laid down by the World Health Organisation (WHO) collection of plant materials [7]. Identification was made, and the voucher specimen was deposited in the Herbarium of Centre of Research for Sustainable Uses of Natural Resources (CoR-SUNR), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia. Fresh sample was cleaned using tap water to eliminate impurities or soil debris. The sample was crushed to a fine powder with an electric blender after shade drying at room temperature and kept in a zip lock bag in a freezer (−20°C) for further analysis [8, 9].

2.2. Sample Extraction. Methanol, ethyl acetate, and hexane solvents were used for the extraction of plant samples by successive maceration methods as described by Bhunu et al. [10] with some modifications. The mixture was filtered using a vacuum filter and then evaporated using a rotary evaporator where the yield of extracts was found; hexane (6.22%), ethyl acetate (8.63%), and methanol (20.6%) were used. The extract was later used to identify its phytochemicals, antioxidant, and antiproliferative effects.

2.3. Determination of Antioxidant Activities

2.3.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. DPPH scavenging activity of the sample was determined according to Bakar et al. [11] and Hassan and Bakar [12]. Briefly, a methanol solution of DPPH (0.3 mM) was added into the samples (2.5 mL). The extract was kept in a light protected place at room temperature for 30 min after mixing vigorously. The absorbance was read at 518 nm. The following equation was used for calculating the scavenging or antioxidant activity (AA):

\[
AA(\%) = \frac{\text{Absorbance (sample)} - \text{absorbance (empty sample)}}{\text{absorbance (control)}} \times 100. \tag{1}
\]

2.3.2. Ferric Reducing Antioxidant Power (FRAP) Assay. FRAP assay was conducted according to Dusuki et al. [13] with some modifications. The FRAP mixture was formed by mixing 300 mM acetate buffer with 3.6 pH, 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, and 0.25 mL of 20 mM FeCl3, and the substances were tested in 0.1 mL methanol, ethyl acetate, and hexane or methanol. Plain reading was recorded at 593 nm using a test tube with extra 3 mL of FRAP reagent. Plant extract (100 µL) and distilled water (300 µL) were supplemented to the test tube. Using a spectrometer, after 30 min of incubation, absorbance was read at 593 nm. Ferric reducing capacity in 1 g of dried sample (mM/g) was measured as mM for the final result. All tests were run in triplicate.

2.3.3. 2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonic Acid (ABTS) Assay. ABTS assay was carried in line with Re et al. [14] with some modifications. Reacting of ABTS solution (7 mM) with 2.45 mM of potassium persulfate produced the preformed radical monocation of ABTS. The solution was kept in the dark and left to stand for 15 h at room temperature. To obtain the units of absorbance at 734 nm, the solution was diluted with either methanol, ethyl acetate, or hexane; aliquot for every sample (200 µL) was added to 2000 µL of ABTS. Using a spectrometer, the absorbance was monitored for 5 min at 734 nm. Blank solvents were run appropriately in every assay. The proportion of inhibition was calculated against control and matched to a vitamin C standard curve (10–100 mm), and the percentage was used to express the radical scavenging activities.

2.4. Anticancer Activities

2.4.1. Cell Culture Condition. MCF-7 and MDA-MB-231 breast cancer cell lines were purchased from American Type Culture Collection (ATCC). The cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 media supplemented with L-glutamine, 1% penicillin-streptomycin,
and 10% fetal bovine serum in a humidified atmosphere of 5% carbon dioxide (CO₂) at 37°C.

2.4.2. Extracts Preparation. To solubilise the plant extract, dimethyl sulphoxide (DMSO) at a concentration of 10mM which was kept at 4°C under light protection was used. DMSO concentration that does not exceed 1% was used for the whole experiment. Fresh complete culture media were diluted with 20 mg/10 mL of standard drug (doxorubicin), and the extract was serially diluted to the cell plates at varying concentrations from 100 \( \mu g/mL \) to 1.5625 \( \mu g/mL \).

2.4.3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide (MTT) Assay. Following a procedure described by Rahmat et al. [15], 6.9 \( \times \) 10⁵ of MDA-MB-231 and MCF-7 cells were plated in 96-well plate and grown in 200 \( \mu L \) complete growth media (CGM) and treated for 24 h, 48 h, and 72 h, respectively. IC₅₀ of the extract was added in 96-well plates in serial dilution (100 \( \mu g/mL \)–5.625 \( \mu g/mL \)), and then, 20 \( \mu L \) of MTT was added into each well and nurtured for 4h. Afterward, to liquefy and solubilize the colored crystals, 100 \( \mu L \) of DMSO was added into each well, and absorbance was read at 570nm using an ELISA reader (AWARENESS-State Fax, USA). The following formula was used to determine the cytotoxicity of the extract:

\[
\text{Cytotoxicity} \% = \frac{\text{optical density of sample}}{\text{optical density control}} \times 100.
\]

For the suppression concentration (IC₅₀), the quality of sample that readily inhibits cell division by half was determined explicitly for every cell multiplying curve.

2.4.4. Cell Cycle Analysis by Propidium Iodide (PI) Staining. Cell cycle analysis was carried out with some modifications as described by Queiroz et al. [16]. Cells (1 \( \times \) 10⁶) were nurtured for 24 h and subsequently treated for 24 h, 48 h, and 72 h using crude extracts at the IC₅₀ values. Altogether, the detached and adhering cells were reaped and reassigned in a disinfected centrifuge tube. The cells were then centrifuged (1,200 rpm) for 15 min at 4°C. Cold phosphate buffered saline (PBS) was used to wash the cells where the cells were resuspended with 0.5 mL of cold PBS, and ice-cold ethanol (70%) was then added to the cell suspension and incubated at −20°C for 2 h. Then, the ethanol was expelled from the sample after centrifugation. The cells that have been rinsed twice using cool PBS previously were stained with propidium iodide (500 \( \mu L \) of 10 \( \mu g/mL \)) and RNase (100 \( \mu g/mL \)) at room temperature for about 30 min. Dispersion of the cell cycle was analysed using flow cytometry.

2.4.5. Annexin V/PI Apoptosis Assay. This method was performed according to Tor et al. [17]. Briefly, cells were plated in 6-well plates and treated with IC₅₀ values for 24 h, 48 h, and 72 h. The viability of the cells was measured using an annexin V-FITC/PI kit, following the manufacturer’s procedure. Both treated and control cells were centrifuged and washed twice using PBS. At room temperature, cell staining was carried out using FITC annexin V (5 \( \mu L \)) and 10 \( \mu L \) PI for 15 min, and cell death was also analysed using flow cytometry.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. The selected crude extract was analysed using gas chromatography (GC-MS-2010 Plus-Shimadzu) in order to determine the secondary metabolites present. The segment (30.0 m length, 0.25mm ID, and 0.25 \( \mu m \) thickness) was set at 50°C for 4 min which was then expanded to 300°C at the rate of 3°C/min, and after that supported for 10 min. The temperature at the injector was set at 250°C, and the volume was set at 0.1 L. The presence of helium rate of the bearer gas was set at 1 mL/min with an aggregate run span of an hour. Electron ionization and mass spectra were performed at 70 eV and between the range of 40–700 m/z [18].

2.6. Statistical Analysis. All the data were presented as mean ± standard error of mean (S.E.M). The data were statistically analysed by one-way ANOVA, followed by Dunnett’s posthoc using Prism version 15.0. The level of statistical significance was set at \( p < 0.05 \).

3. Results

3.1. Antioxidant Activity of the Plant Extracts. Since one antioxidant assay is not sufficient to provide an adequate picture of the scavenging activities, \textit{D. bulbifera} leaf extracts were subjected to several antioxidant assays. Antioxidant
3.2.1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide (MTT) Assay. Cytotoxic activity of the plant extracts against the breast cancer cell lines was assessed using MTT assay. The IC$_{50}$ values of extracts on the viability of cells were determined which lower IC$_{50}$ values signifying a higher antiproliferative activity. All the three extracts tested have demonstrated significant and effective antiproliferative activities in both dosage and time-dependent manner.

3.2.2. Cell Cycle Arrest. Using the IC$_{50}$ concentration from MTT assay for 24h, 48h, and 72h, cell cycle analysis was evaluated after exposure of D. bulbifera methanol extract (Figure 2). In MCF-7, there was a momentous arrest at sub-G$_1$, at 24h–72h, and the number of cells at S and G$_2$/M phases and the number of cells at G$_0$/G$_1$ were reduced substantially. In MDA-MB-231, there was a significant arrest at sub-G$_1$ and G$_2$/M for 24h, 48h, and 72h, and the number of cells in G$_0$/G$_1$ and the number of cells in G$_2$/M were markedly reduced. At 48h, there was also considerable arrest at sub-G$_1$ and G$_0$/G$_1$, and the number of cells in S and the number of cells in G$_2$/M phases were markedly reduced, and eventually at 72h, there was a substantial arrest at sub-G$_1$ and S, and the number of cells in G$_0$/G$_1$ and G$_2$/M phases decreased significantly.

3.2.3. Apoptosis. Cell lines were treated, and the IC$_{50}$ values for 24h, 48h and 72h were obtained. The cells were stained with both FITC-conjugated annexin V and PI. FACS was utilised to acquire the stained cell populace. Histograms from FACS investigation at each concentrate fixation are shown in Figure 2. Graphical presentation (Figure 3) shows the proportion of annexin-V-FITC and PI-stained cells at each phase. Histograms from FACS investigation at each concentrate fixation are shown in Figure 2. Graphical presentation (Figure 3) shows the proportion of annexin-V-FITC and PI-stained cells at each phase.

### Table 1: Antioxidants activities of D. bulbifera leaves extract.

| Sample name     | DPPH (%) | FRAP (mM/g) | ABTS (mg AEAC/g) |
|-----------------|----------|-------------|------------------|
| Ascorbic acid   | 97.0 ± 0.21 | 76.5 ± 0.5 | 47.1 ± 0.81 |
| Methanol extract| 79.0 ± 0.31 | 65.6 ± 0.35 | 31.34 ± 2.06 |
| Ethyl acetate extract | 23.2 ± 0.05 | 59.5 ± 0.10 | 10.98 ± 0.64 |
| Hexane extract  | 11.5 ± 0.31 | 14.9 ± 0.05 | 9.50 ± 0.48 |

The IC$_{50}$ values of the extract on the viability of cells in MCF-7 after 24h, 48h, and 72h of incubation were 41.17 μg/mL, 15.71 μg/mL, and 11.53 μg/mL, respectively, while the standard drug, doxorubicin, has shown the potent antiproliferative effect on the tested cell line with the IC$_{50}$ values of 5.87 μg/mL, 3.23 μg/mL, and 1.98 μg/mL for 24h, 48h, and 72h, respectively. For the MDA-MB-231 cell line, the methanol extract of D. bulbifera had IC$_{50}$ values of 4.29 μg/mL, 1.86 μg/mL, and 1.23 μg/mL, respectively, at 24h, 48h, and 72h. On the other hand, doxorubicin displayed potent cytotoxicity against the tested cell line with IC$_{50}$ values of 11.21 μg/mL, 8.1 μg/mL, and 3.07 μg/mL for 24h, 48h, and 72h, respectively.

The findings showed that the plant extracts had substantial disparities in their ability to compare ascorbic acid, which may also be ascribed to various characteristics, rea,ction, and mechanisms. Organic media can be used in solubilising DPPH radical chromogens. A strong positive correlation ($R^2=0.9399$, $p<0.05$) was observed between the activities. There was also a similar relationship in FRAP, where a strong positive linear correlation ($R^2=0.8143$, $p<0.05$) was found. FRAP also correlates with ABTS* + ($R^2=0.87$), whereas ABTS*+ showed a stiff positive association to VCEAC ($R^2=0.6784$). The practical difference in these comparisons was DPPH*, which also correlates poorly with ABTS ($R^2=0.53$).
Figure 2: Flow cytometric scans of treated and untreated (control) cells.

Figure 3: Annexin V FTIC of treated and untreated (control) cells.
increased by 8.02%. Although the proportion of cells in the group of live cells decreased significantly by 28.43% in MDA-MB-231 after 72 h of treatment, the percentage of cells in early apoptosis increased by 29.37%. Similarly, at the necrotic level, the proportion of cells increased by 41.46% but decreased by 0.74% in the late apoptotic period. However, for the control culture at 72 h, the proportion of cells with early apoptosis decreased by 4.14%, the late apoptotic stage also decreased by 14.62%, while the apoptotic period increased by 1.08%, and a number of viable cells also decreased by 11.92%.

3.3. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis. GC/MS investigation was conducted on D. bulbifera methanol extract. The peaks in the chromatogram were integrated and matched with the database spectra of recognised compounds stored in the GC-MS libraries of Pfleger–Maurer–Weber-drug, National Institute of Standard and Technology (NIST), WILEY229.LIB, Flavour, Fragrance, Natural and Synthetic Compounds (FFNSC1.3.lib), and Pesticides Library for toxicology (PMW_tox2).

The result of D. bulbifera methanol extract revealed 50 peaks, with 45 compounds identified, representing 98.49% of the entire extract (Figure 4). The major among them were acetic acid (34.68%), n-hexadecanoic acid (14.89%), 1,2,3-propanetriol, 1-acetate (acetin) (7.28%), hexadecanoate <methyl-> (4.01%), 7-tetradecenal (Z) (2.92%), glycerol alpha-monoacetate (2.80%), phytol (2.46%), octadecanoic acid (2.26%), cholesterol (2.10%), palmitic acid (1.35%), linolenate <methyl-> (1.32%), megastigmatrieneone and 8-oxabicyclo-oct-5-en-2-ol, 1,4,4-trimethyl (1.28%) each, 1,2,3-propanetriol (1.23%), and 4,4,5,8-tetramethylchroman-2-ol (1.22%).

4. Discussion

Medicinal plants have been of great interest as a source of natural antioxidants used for health promotion such as anticancer properties. In this present study, the radical scavenging activities of D. bulbifera leaf extracts were quantified using DPPH, FRAP, and ABTS assays. All the extracts demonstrated scavenging of stable DPPH and ABTS as well as reducing activity in FRAP assay. The secondary metabolites, namely, phenolics and flavonoids, present in this species make it potential for antioxidant activities by functioning as reducing agents [15, 19–22]. Numerous antioxidants that are described to have therapeutic potential such as vanillic acid, isovanillic acid, epicatechin, and myricetin are essential bioactive compounds in D. bulbifera. Tubers of D. bulbifera have displayed higher scavenging activity, reducing power, and ferrous ion chelating due to its high content of polyphenols such as oxalic acid, citric acid, malic acid, and succinic acid. Thus, the secondary metabolites might be the key role players behind its biological activities [23]. The methanolic extract of D. bulbifera has been reported to demonstrate DPPH radical-scavenging activity [24]. Studies of antioxidants from different platinum–palladium bimetallic nanoparticles (PtNPs) made from D. bulbifera indicates that PtNPs could inhibit radical DPPH by up to 30.16%, while palladium (PdNPs) showed up to 28.97% of the activity. Despite this, Ptfor–PdNPs reveal 38.49% scavenging when compared separately with PtNPs and PdNPs. In addition, for Pt–PdNPs (56.71%), a synergistic enhancement activity against radical superoxide was also observed as contrasted and only PtNPs (31.87%) or PdNPs (27.1%).

Another study which was conducted by Li et al. [25] revealed that D. bulbifera and other Dioscorea species possessed high antioxidant activity as in the following order D. bulbifera, followed by D. colletitii, D. nipponica, and D. opposita. This indicates that members of the genus Dioscorea have therapeutic potential due to high antioxidants contents. Comparable study was carried out by Ghosh et al. [26]; copper nanoparticles (CuNPs) synthesised by D. bulbifera showed high radical scavenging activities that are slightly lesser when compared with ascorbic acid. Vitamin C also showed 14.11% of superoxide scavenging activity while CuNPs showed 48.39% of the activity.

The results of the present study indicated that the methanolic extract of D. bulbifera was found to be the most cytotoxic extract in both cell lines due to the presence of plentiful active compounds, namely, diosgenin in yam, the
edible tubers of *Dioscorea* spp. These results are compatible with the studies conducted by Li et al. [27] and Lee et al. [28]. Diosgenin has been shown to enhance metabolism of lipids, improve antioxidant activities, reduce glucose levels, and suppress inflammation [29]. Previous research presented that foods rich in diosgenin such as yam species (known as air potatos) found to have a protective effect on myocardial I/R wound in rats due to apoptosis and necrosis and showed a cytotoxic effect on cancer cell lines [29–34].

It was reported by Nur and Nugroho [32] that *D. bulbifera* extracts demonstrated a cytotoxic effect on T47D cell lines. This research result indicated that the chloroform extract of the *D. bulbifera* leaves was stronger in inhibiting the growth of the cells than the methanol extract. Similarly, Ghosh and his colleagues [26] revealed that different nanoparticles Ptn–PtNPs, PdNPs, and PtNPs made from *D. bulbifera* tuber extract exhibited the anti-proliferative effect where Pt–PdNPs had been the most cytotoxic nanoparticles at concentration 10 μg/mL. In *vivo* studies conducted on cytotoxic activities of the water extract, nonethyl acetate extract, ethyl acetate extract, ethanol extract, and diosbulbin B isolated from *D. bulbifera* showed that ethanol and ethyl acetate extracts reduced the lump weight in S180 and H22 tumour cells bearing mice while no such effect was observed in water and nonethyle acetate extracts [35].

In this study, cell cycle analysis was conducted in MCF-7 and MDA-MB-231 cell lines after treated with *D. bulbifera* methanol extract at IC50 concentration for 24h, 48h, and 72h. At 24h, the extract induced S and G2/M phases arrest in MCF-7 and G0/G1 and G2/M phases arrest in MDA-MB-231. At 48h, growth in a number of the treated cells was observed in the sub-G1 phase in MCF-7 and sub-G1 and G2/G0 in MDA-MB-231, while significant decreases in the number of cells were observed in G0/G1, S, and G2/M in MCF-7 and S and G2/M in MDA-MB-231. At 72h, *D. bulbifera* methanol extracts arrested MCF-7 at the sub-G1 phase and MDA-MB-231 at sub-G1 and S phases.

The result of the present study was similar with Srinivasan et al. [36] where diosgenin led to cell growth in the G1 phase, consisting of 72% of cells in G1 at 24h and 82% and 76% at 48h and 72h posttreatment, respectively, compared to untreated cells. Similar observation was found in diosgenin-treated MDA-MB-231 cells where there was aggregation of cells in the G1 process occurred (82% at 24h, 81% at 48h, and 71% at 72h). In addition, Moalic et al. [37] found that cells treated with diosgenin suppressed 1547 cell proliferation rate after 12h along with a significant accumulation of cells in the G1 phase which was increased at 24h. Consequently, the fraction of S phase cells decreased at 12h. A sub-G1 population, typically associated with apoptotic cells, appeared at 48h when compared to controls. According to a similar study conducted by Wang and Weller [38], HepG2 cells treated with different concentrations of protodioscin (bioactive component in *D. colletti*) for up to 24h, accumulated mainly in the G2/M phase in a dose-dependent and time-dependent manner with consequent increase in the sub-G1 phase of cell cycle. Moreover, diosgenin isolated from *D. bulbifera* was reported to induce S-phase arrest at a concentration of 13 μM in DU-145 prostate cancer cells. At 24h of incubation phase, S arrest was relatively substantial, but at 48h of incubation, there was no such effect, and arrest was not found to be important in MCF-7 compared to DU-145 [39].

In 2005, Liu et al. [40] reported that NB4 cells treated with diosgenin resulted in the enlargement of cell size, and arrest was made at G2/M. Diosgenin increased the p53 levels in NB4 cells, indicating its importance in controlling cell cycle arrest. Flow cytometric sub-G1 analysis showed that a dramatic hypodiploid number of K562 cells appeared after treatment with diosgenin for 48h along with DNA fragmentation. Another research carried out by Hsu et al. [41] presented that treatment of squamous cell carcinoma-25 (SCC-25) with red mold *Dioscorea* cell cycle for 24 h caused arrest at the G2/M phase. This effect was also connected to the repression of CDK1 and cyclin B1 mRNA levels, ensuring cell proliferation inhibition.

On comparative research by Liu and his colleagues [40], it was found that dioscin fundamentally hinders multiplication of C6 glioma cells at the S stage because of an expansion in a few cells in the stage following expanding dosages of dioscin increment in sub-G1 arrest. Demonstrating that dioscin caused cell cycle capture at the S stage, G0/G1 stage decline in a few cells was observed. It has been recently detailed that dioscin restrained ROS creation, brought about DNA harm, and made cell cycle captured at the S stage, when contrasted and untreated, gathering the extent of the G0/G1 stage decreased from 61.38% to 35.43%, and S stage increased from 30.62% to 59.77% by 5.0 mg/ml of dioscin while the extents of G2/M stage had small changes. These findings showed that dioscin was able to block human HEp-2 and TU212 cells at the S stage [42].

A study performed by Li et al. [27] showed that diosgenin treatment in focus subordinate caused increase of G2/M stage cell population in Bel-7721, SMMC7721, and HepG2 HCC cells, suggesting that diosgenin could arrest the cell cycle in the G2/M stage because of the way that extent of G2/M stage cells with expanded in centralization of diosgenin. Lee and his colleagues [28] presented the anti-proliferative impacts of diosgenin from yam (*D. pseudojaponica*) on malignant growth (MCF-7, A 549, and Hep G2) and typical (HS68 and clone 9) cells. The outcome demonstrated that diosgenin from *D. pseudojaponica* hindered MCF-7 cells multiplication through G0/G1 arrest.

The importance of the apoptosis idea for oncology lies in its being a directed marvel subject to incitement and hindrance. Even though little was thought about how settled helpful operators for disease influence its introduction, it appears to be sensible to propose that more prominent comprehension of the procedures included may prompt the advancement of enhanced treatment regimens [43]. Apoptosis is a physiological process of cell death that is in charge of the destruction of cells in normal tissues; it likewise occurs in different pathologic settings. Morphologically, it involves quick build up and sprouting of the cells with the arrangement of membrane-enclosed apoptotic bodies containing well-preserved organelles which are phagocytosed.
and digested by nearby resident cells [44]. Generally, apoptosis involved two central pathways; the first one is the stimulation of death receptors (DRs) in the tumour necrosis factor (TNF) superfamily, and the second one is the mitochondrial pathway initiated by Bcl-2 family proteins [45].

Several drugs that are used for cancer treatment have been appeared to cause apoptosis in quickly dividing normal cell numbers and tumours. Consequently, enhanced apoptosis is liable for a significant number of the antagonistic impacts of chemotherapy and tumour regression. Clear understanding of the procedures involved might lead to the improved treatment regimens. Hence, the realization of anticancer drugs that mediated the therapeutic impact by activating apoptosis is an additional necessary outcome [44].

Annexin-V-FITC/PI-flow cytometry analysis ascertained the induction of apoptosis by plant crude in both MCF-7 cells and MDA-MB231. Distinctive biochemical features characterise apoptosis in the removal of damaged cells or tumour cells without causing irritation. The commencement of enzymatic and catabolic procedures in apoptosis in this manner empower cell morphological changes, for example, externalization of the plasma layer, phosphatidylinerse (PS), shrinking of the cells, blebbing in cell membrane, condensation of chromatin, nuclear fragmentation, and apoptotic bodies formation [46–49].

5. Conclusion

In conclusion, the effort presented herein has proved that D. bulbifera methanol extract had resilient antiproliferative activity when compared with a standard drug. The antioxidant activities shown by methanol, ethyl acetate, and hexane extracts of the plant were significant compared with ascorbic acid, indicating the potential of the leaves of this species as natural antioxidants. Hence, antiproliferative activities demonstrated by leaf extracts authenticate the old style uses of this plant against various diseases of breast cancer inclusive.

Data Availability

The datasets generated and/or analysed during the current study are available from the first author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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