

Research Article

Antimetastatic potential of anthocyanins from *Cordyline australis* (G. Forst.) Endl. Red star variety on MCF onco cell lines

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Abstract

Breast cancer is the second most deadly diagnosed lifestyle disease among women. Surgery and chemotherapy are the current treatments of choice; nevertheless, toxicity connected with this underscores the urgency of the demand for the human-friendly drug. 50% of current synthetic drugs available commercially today are either direct or indirect descendants extracted from herbs. Anthocyanins possess many pharmacological activities, including anticancer potential. However, no study on anticancer activity of anthocyanins from *Cordyline australis* has been reported. Anthocyanins were extracted from fresh leaves using ethanol as solvent. The total anthocyanin was quantified and fractionated by Ultra Performance Liquid Chromatography. Cytotoxicity effect was carried on diverse cancer cell lines like MCF 7, HCT-116, Caco-2 and SW480 using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay). Based on MTT data, MCF 7 cells were further analyzed by LDH assay, Glutathione-S- transferase (GST), Quercetin reductase, Cytochrome P450 and Caspase 3, 8 and 9 activities. The obtained results were analyzed using ANOVA with a level of significance. Results obtained from MTT assay revealed that the anthocyanin extract carried significant toxic ($p < 0.05$) specificity against MCF 7 cells (65 ± 2.1 toxicity at $50 \mu\text{g/ml}$) when compared to the other onco cells. Remarkable LDH leakage (50.2% vs $50 \mu\text{g/ml}$), GST (3.0 ± 0.002 U/mg protein), QR (4.4 ± 0.054 U/mg protein), Cyt P450 activities (0.291 ± 0.01 U/mg protein) were noticed. Caspase 3 (157%), 8 (142%) and 9 (147%) displayed profound activities. These *in vitro* findings of specific anticancer effects noticed on *C. australis* anthocyanin extract require further evaluation using animal models. Finally, the obtained findings open up the possibility of developing a lead antimetastatic anthocyanin candidate against deadly breast cancer.

Keywords: Antimetastatic, Anthocyanin, Caspase, Cytochrome P450, Glutathione-S- transferase (GST), Lactic dehydrogenase, Quercetin reductase

INTRODUCTION

Breast cancer is one of the most prevalent types of cancer, with the highest incidence among all cancer types that occurs in women. Despite advancements in research, screening and treatment, the mortality rates associated with it remain high and metastasis is the leading cause of mortality (Zhou *et al.*, 2017). The study of phytochemicals extracted from fruits, vegetables and herbals has been focused, indicating that phytonutrients have a significant role in oncotherapy (Han, 2005). Anthocyanins are the most abundant flavanoid constituents in vegetables and fruits. They are water-

soluble and their occurrence is ubiquitous in the plant world (Wang and Stoner, 2008; Liu, 2021).

Over the years, researchers have revealed that anthocyanins are non-toxic natural pigments with antioxidant and anti-inflammatory potentialities. The conjugated bonds present in anthocyanin structures absorb light at 500 nm wavelength and are responsible for diverse colours such as blue, bright red and purple found in berries, grapes, apples, purple cabbage and corn (Wang and Stoner, 2008). Anthocyanins also possess antimicrobial, antiviral, antiallergic, anticarcinogenic, antimutagenic, and antiproliferative effects and therefore play significant roles in preventing and lowering

the risk of cardiovascular disease, diabetes, arthritis and various cancers (Liu, 2021; Diaconeasa, 2018).

Anthocyanins have exhibited *in vitro* and *in vivo* anticarcinogenic efficacies against multiple cancer cells. Their chemopreventive activities include radical scavenging, stimulation of phase II detoxifying enzymes, reduction in cell proliferation, angiogenesis & invasiveness, and induction of apoptosis and differentiation (Wang and Stoner, 2008). Anthocyanins with *ortho*-dihydroxyphenyl, on their B-rings possess the most obvious anticancer potentiality by targeting RTKs (EGFR, PDGFR and VEGF/VEGFR) / Ras-MAPK and PI3K/Akt signal cascade pathways, inhibit the cancer cell growth and metastasis. Similarly, anthocyanins regulate the functionality of gene expression through apoptotic pathways.

Anthocyanin extract provides an adjuvant therapeutic effect for breast cancer. According to Tan (2020), anthocyanins from the skin of grapes increase intracellular ROS levels leading to apoptosis in MCF-7 breast cancer cells and also arrests the cells in the G2/M phase. Mazzoni (2019) reported that anthocyanin extract from Alba strawberry induces apoptosis and cell death of breast cancer cells by downregulating AMPK expression and lowering breast cancer. Li (2009) reported that fruit extracts of *Eugenia jambolana*, contain 3.5% anthocyanins, which causes proapoptotic effects only on breast cancer cells and not on normal breast cells. Cyanidin-3-O-sam, the anthocyanin fraction obtained from the fruits of the deciduous shrub, *Acanthopanax sessiliflorus*, inhibits the metastasis of breast cancer cells by causing the suppression of neovascularization and the gelatinolytic potential of MMP-9 (Lee, 2013). In this scenario, the present study attempted to evaluate the anti-metastatic efficacy of anthocyanins from Red star variety of *Cordyline australis*.

MATERIALS AND METHODS

Plant material

C. australis (G.Forst.) Endl. (Figure 1) is an erect monocot shrub endemic to New Zealand. The height varies from 5-8m, leaves are narrow and linear with bronzed-red colour. Upper leaves are erect, while the lower leaves are horizontal and drooping, forming star burst-shaped clusters. Leaves of Red star variety of *C. australis*, collected from Thiruvananthapuram District of Kerala, were used for the present study.

Assay of total anthocyanin content (TAC)

Anthocyanins were isolated with ethanol and quantified as per the method of Young and Abdel-Aal (2010). The absorbance was read at 520 nm against distilled water as the blank. The data were expressed as μg of cyanidin 3-glucoside (Cy 3-glu) equivalents / g of fresh weight.

Fractionation and quantification of anthocyanin by Ultra Performance Liquid Chromatography (UPLC)

Sixteen anthocyanin standards such as cyanin chloride (cyanidin-3,5-di-o-glucoside chloride), delphinidin chloride (3,30,40,5,50,7-hexahydroxyflavylium chloride), cyanidin-3-o-glucoside chloride, delphinidin chloride (delphinidin-3,5-di-o-glucoside chloride), cyanidin-3-o-rutinoside chloride, delphinidin-3-o-glucoside chloride, delphinidin-3-o-sambubioside chloride, delphinidin-3-o-galactoside chloride, delphinidin-3-o-rutinoside chloride, malvin chloride (malvidin-3,5-di-o-glucoside chloride), malvidin-3-o-glucoside chloride, peonidin-3,5-di-o-glucoside chloride, peonidin-3-o-glucoside chloride, petunidin-3-o-glucoside chloride, peonidin-3-o-rutinoside chloride and pelargonidin 3-o-glucoside chloride were used for the study. 1 mg/mL stock solutions for all the standards were prepared and standard curves in the concentration range between 1 to 200 ppm were made. The extract was concentrated using a rotavapor at 37 °C and the dried extract was dissolved in 1 mL methanol. UPLC of these anthocyanin extracts was performed using Waters Acquity Ultra Performance. LC system, equipped with a quaternary pump system following the protocol of Sharma *et al.*, 2020. Eluent A comprised 5% (v/v) formic acid and eluent B comprised HPLC grade acetonitrile. Gradient elution was employed to carry out separation for 6.8 min. The extraction and quantification of anthocyanins were performed by comparing the retention times of anthocyanin peaks in the sample extracts with the standards and calibration curves.

Cells and culture conditions

Human cancer cell lines such as MCF 7, HCT-116, Caco-2 and SW480 (ATCC) were purchased from National Centre for Cell Science (NCCS), Pune and were maintained with Dulbecco's Modified Eagle Medium (DMEM) medium as specified above. On the day before the treatment, the cells were seeded in six-well plates at a density of 5×10^5 . Then the cells were replaced with fresh DMEM with & without 10% FBS (serum-free conditions) and then treated with 10, 20, 30



Fig. 1. Plant material *Cordyline australis* (G.Forst.) Endl.

and 50 µg/ml of anthocyanin extracted from the leaves of *C. australis*.

Cytotoxicity and apoptosis assay

Cytotoxicity was assessed by MTT reduction assay as per the protocol of Greeshma *et al.*, 2020. Cancer cells were isolated and digested using trypsin into single cell suspension during the logarithmic phase of growth. They were seeded into the 96-well plate at a 2.0×10^4 per well density. Cells were divided into three groups: blank control group (culture medium only), control group (cells without extract treatment) and treatment group (cells treated with various doses of extracts). After 24 h, various concentrations of anthocyanin extracts were added and incubated with the cells. After another 24 h, 20 µl 5 mg/ml MTT solution was added to each well and incubated for 4 h. DMSO (100 µl) was added following the removal of the supernatants. The crystals were thoroughly dissolved and the absorbance value of each well was measured at 570 nm by enzyme-linked immunosorbent assay (ELISA). The % of inhibition was calculated as $\% = (1 - \text{average absorbance value of the treatment group}) / \text{average absorbance value of the control group} \times 100\%$.

Lactate dehydrogenase (LDH) assay

Lactate dehydrogenase (LDH) cytotoxicity assay kit was used to evaluate the cell membrane damage. The assay was based on the activity of LDH leached from the damaged cells into the medium (Kaja, 2017). Cells were plated in the 96-well plates (1×10^4 cells / well) and incubated for 24 h. To the cells with different dosages (10, 20, 30 and 50 µg/ml), *C. australis* anthocyanin extract was introduced and incubated for another 24 h. 1% Triton of lysis solution was added to the control cells at 45 min prior to the centrifugation and was selected as positive control. Centrifugation was carried out at 1200 rpm for 5 min and 100 µL of the supernatant from each well was transferred to 96-well culture plate and OD was read at 490 nm. The LDH leakage % of positive control was recorded as $\% \text{ of } (OD_{\text{test}} - OD_{\text{blank}}) / (OD_{\text{positive}} - OD_{\text{blank}})$, where OD_{test} is the OD of the control cells or cells exposed to anthocyanin, OD_{positive} is the OD of the positive control cells and OD_{blank} is the OD of the wells without cells.

Isolation and assay of enzymes

The treated and control MCF 7 cells were homogenized at 4°C in phosphate buffer (pH 7.0, 0.1 mol/L potassium phosphate) using hand held homogenizer. Subsequently, the cells were centrifuged for 20 min at 4°C and 10,000 X g. The supernatant was re-suspended in phosphate buffer.

Glutathione-S- transferase (GST) activity was recorded in the cytosol with Spectrophotometer as per the method of Habdous *et al.* (2003) using 10 mmol/L 1-chloro-

2,4-di nitrobenzene (CDNB) as substrate. In the reaction mixture, incubation of 0.2 to 30 mg tissue cytosol protein was carried out briefly. The reaction mixture attained a final volume of 1 ml and included 0.1 mol/L potassium phosphate buffer (pH 6.5), 6.2 mmol/L glutathione, and 10 mmol/L CDNB. The reaction was initiated upon the addition of CDNB. The velocity of formation of S-(2-chloro-4-nitrophenyl) glutathione was noted for every 30 sec for 3 min. at 340 nm, at 25°C. One unit of enzyme activity = 1 nmole conjugate formed/min/mg protein. The molar extinction coefficient for CDNB was 9.6 nmol/ml.

Quercetin reductase (QR) activity in the cytosol was assayed using 12 mmol/L DPIIP (2,6-dichloroindophenol) as substrate (Prochaska and Santamaria, 1988). The total volume in the cuvette included 0.06 to 0.28 mg tissue cytosol protein incubated with 25 mmol/L Tris-HCl (pH 7.4), 0.7 mg BSA, 1% Tween 20, 5 µmol/L FAD, and 0.2 mmol/L NADPH and 0 or 10 µmol/L dicoumarol at 25°C, to which 40 µmol/L DPIIP was added to start the reaction. The reduction of DPIIP was measured at 600 nm, for 3 min, with readings taken every 15 sec. The dicoumarol sensitive part of the activity was taken as a measure of the QR activity. Cytochrome P450 (CYP1A1) activity was evaluated in the cells as per Manson *et al.* (1997) method with 7-O-ethoxyresorufin as the substrate. Total volume of the reaction mixture was 3 ml, which includes 25 µl cell protein, 0.1 mol/L potassium phosphate buffer (pH 7.4), 50 mmol/L NADPH and 0.75 mmol/L ethoxyresorufin. The sample buffer and ethoxyresorufin were incubated at 37°C in a water bath for 4 min with shaking at low speed. After 4 min, 100 mmol/L NADPH was added to initiate the reaction. The formation of resorufin was read at an excitation wavelength of 550 nm and emission wavelength of 581 nm, for 2.3 min. at 20°C. The protein concentration of each tissue was measured using the method of Lowry *et al.* (1951). Data were expressed as U/mg protein.

Caspase-3, -8, and -9 colorimetric assays

Quantitative estimation of human caspase-3, 8, and 9 was carried by Caspase-family Colorimetric Substrate Set Plus as per the manufacturer's (Biovision, Milpitas, CA, USA) instructions. Cells (10^6 /ml) were incubated with different doses of BAA extract (IC_{10} , IC_{20} , and IC_{50}) for 24 h before treatment (Pu, 2017). The treated and untreated cells were harvested and centrifuged at 1000 rpm for 5 min and the pellets were lysed by adding a cold lysis buffer and incubated on ice for 10 min. After that, the cells were again centrifuged at 5000 rpm for 2 min and from the lysate 50 µl was transferred to a microplate; 50 µl 2x reaction buffer containing 10 Mm DTT was added to the reaction mixture followed by 5 µl of caspase p-nitroaniline (pNA) substrate, then each well was incubated at 37°C for 1–2 h. The absorbance

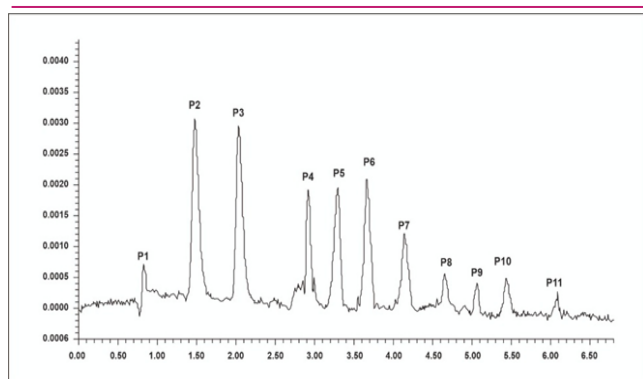


Fig. 2. Ultra performance chromatography (UPLC) chromatogram

of coloured product was noted on a FLUO star Omega microplate reader at a wavelength of 405 nm.

Statistical analysis

Results were analyzed using version 7 of GraphPad Prism, using ANOVA, and differences were considered statistically significant at the level of p-values ≤ 0.05 .

RESULTS AND DISCUSSION

Anthocyanins and anthocyanin-rich plant extracts have exhibited antimetastatic activity against multiple cancer cell types under *in vitro* conditions (Erikade Arruda Nascimento *et al.*, 2022). Cell proliferation was arrested by the anthocyanins by inhibiting various cell cycle stages (inhibits cell cycle regulator proteins like p53, p21, p27, cyclin D1, cyclin A, etc.) (Anantharaju *et al.*, 2016). Many researchers studied the antimetastatic potentials of anthocyanins on normal vs. cancer cells and found that they selectively block the growth of cancer cells with a relatively marginal effect on the growth of normal cells (Zorita Diaconeasa *et al.*, 2020).

The total anthocyanin content of the ethanolic extract from *C. australis* was 27.4 mg/ml. UPLC analysis (Fig. 2) showed characteristic 11 anthocyanin peaks with remarkable level of delphinidin-3-o-galactoside (P2- 29.2 ppm) followed by delphinidin-3-o-glucoside (P3- 25.7 ppm), cyanindin-3-o-glucoside (P4- 20.4 ppm), and cyanindin-3-o-rutinoside (P5- 18.2 ppm). Delphinidin-3-o-rutinoside (P6-18.65 ppm), peonidin-3, 5-di-o-glucoside (P7), petunidin-3-o-glucoside (P8), pelargonidin 3-o-glucoside (P9), peonidin-3-o-glucoside (P10), peonidin-3-o-rutinoside (P11) and malvidin-3-o-

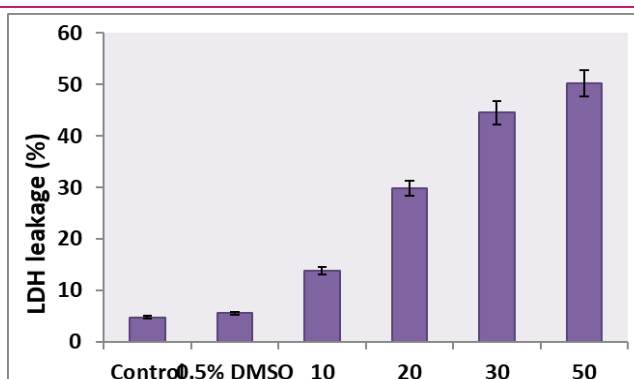


Fig. 3. LDH leakage (%) induced in MCF 7 cells by the anthocyanin extract

glucoside (P1) were the minor peaks recorded.

MTT assay

The antimetastatic potential of anthocyanin extract was done by MTT assay on the selected cancer cell lines such as MCF 7, HCT-116, Caco-2 and SW480. The anthocyanin extract displayed concentration-dependent inhibition of cell proliferation on MCF 7 cell lines. No significant inhibition/cytotoxic impact was noticed with HCT-116, Caco-2 and SW480 onco cell lines. The IC_{50} value of MCF-7 cells was found to be 28.4 ± 0.32 $\mu\text{g/ml}$ (Table 1).

LDH leakage assay

Generally, the cell membranes damage by chemicals/ drugs leads to intracellular leakage of LDH molecules into the culture medium. Thus, the leakage of LDH indirectly reflects the cell membrane integrity, which may be associated with necrosis. Necrosis refers to cell death that will provoke an inflammatory response in surrounding cells by leaking intracellular contents (Adigun *et al.*, 2021). As shown in Fig. 3, the LDH leakage % induced in MCF-7 cells remained 13.8 % at a lower dosage (10 $\mu\text{g/ml}$) compared with a higher concentration of 50 $\mu\text{g/ml}$ (50.2 %). The values were statistically significant at 1%.

The present data revealed that the cells showed morphological deformities of apoptosis rather than necrosis. Helm *et al.* (2017) noted that the drastic loss of membrane integrity may not be used as an index to differentiate apoptosis from necrosis cell death. Meanwhile, Forkasiewicz *et al.* (2020) documented the direct involvement of LDH over expression and subsequent

Table 1. Percentage of inhibition on selected onco cell lines by the anthocyanin extract of *Cordyline australis*

Cell lines	10 $\mu\text{g/ml}$	20 $\mu\text{g/ml}$	30 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$
MCF 7	11.98 \pm 0.59	37.3 \pm 1.08	54 \pm 1.7	65 \pm 2.1
HCT-116	0.061 \pm 0.04	0.095 \pm 0.02	0.11 \pm 0.003	0.12 \pm 0.006
Caco-2	0.04 \pm 0.003	0.08 \pm 0.004	0.1 \pm 0.005	0.1 \pm 0.001
SW480	0.062 \pm 0.007	0.099 \pm 0.001	0.1 \pm 0.002	0.1 \pm 0.007

outbreak of apoptosis.

Quinone reductase (QR), glutathione-S- transferase (GST) cytochrome P450 assay

C. australis anthocyanin extract displayed significant impacts on QR activity i.e. concentration-dependent relationship in MCF 7 cell lines when compared to the control (Table 2) ($p < 0.05$) i.e., from 1.01 ± 0.002 (control) to 4.4 ± 0.054 U/mg protein at 50 $\mu\text{g/ml}$. Similarly, glutathione S-transferase activity (GST) also enhanced above the mean control activities (0.54 ± 0.004 - 3.0 ± 0.002 U/mg protein). Cytochrome P450 also showed a dose dependent performance i.e., the control activity level was 0.15 ± 0.001 , while at 50 $\mu\text{g/ml}$ it was increased to 0.291 ± 0.01 U/mg protein.

Generally, polyphenols inhibit the CYP1A1 and CYP1A2 activities, thereby protecting the cells from carcinogenic activators. In the present study, anthocyanins activated phase I and II metabolizing enzymes such as Quinone reductase (QR), glutathione-S- transferase (GST) cytochrome P450 and there by regulated the metastasis in MCF 7 cell lines (Table 2). Seelinger *et al.* (2008) reported the role of luteolin against tumors via inhibiting angiogenesis. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) belongs to TNF cytokine category. TRAIL was used to evaluate antimetastatic potential. The binding of TRAIL, TRAIL-R1, the death receptor 4 (DR4) and TRAIL-R2 (DR5) triggers the apoptotic signals through the formation of trimerization of TRAIL-R1 and/or TRAIL-R2 (death-inducing signaling complex) leading to recruitment of caspase-8 to the intracellular death domain (DD) of the receptors, which induces a caspase cascade and finally apoptotic death of malignant cells (Manoj, 2012). In the present study, 4, 6 and 2 folds increase in the activity of QR, GST, CYP 2 A1 in MCF 7 cells were observed when compared to control (Table 2). In most antimetastatic studies, phase I and II metabolizing enzymes have not been evaluated to confirm their efficacy against cancer cell lines (Choudhari Amit *et al.*, 2020). This is the first report on *C. australis*, anthocyanin vs QR, GST, CYP 2 A1 enzyme activities. Remya Krishnan and Murugan (2013) reported the potentiality of flavonoids from the liverwort *Marchantia* in the above

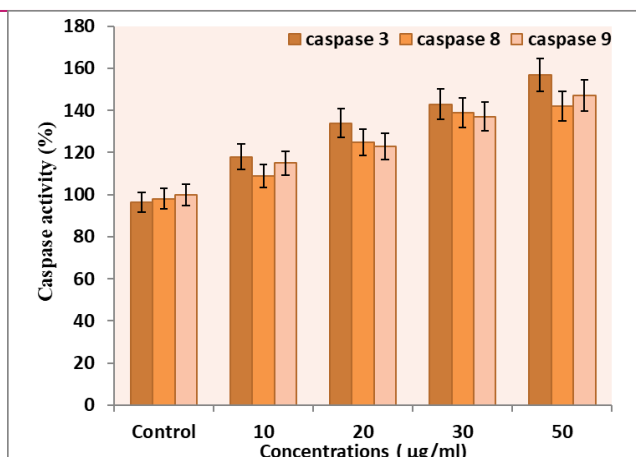


Fig. 4. Caspase 3, 8, 9 activities (%) induced in MCF 7 cells by the anthocyanin extract of *Cordyline australis*

enzymes in SW 480 cells. The present data was almost at par with the activity displayed by flavonoids of liverworts.

Induction of caspase activity

The hallmark of apoptosis occurs through the activation of the caspases and tracing its pathway induced by antimetastatic drugs may offer crucial input in modeling better treatment strategies in cancer. In the present study, the activation of caspase 3, 8, and 9 was analyzed at 24 h with various dosages of anthocyanin extract. MCF7 cells, following exposure to anthocyanin extract, showed profound elevation of effector caspases - caspase 3 activities at 30 and 50 $\mu\text{g/ml}$ concentrations. In addition, the anthocyanin extract was able to induce caspase 8 activity. Interestingly, the caspase 9 initiator caspase activity was also increased in MCF7 cells treated with anthocyanin (Fig. 4).

The two basic reactions involved in apoptosis are intrinsic (mitochondrial) and extrinsic (death receptor) pathways. The effector caspases - caspase-3/7 is involved in the final execution of death, while caspase 8 and 9 are initiator caspase regulates the intrinsic pathway. Caspase 3, 8 and 9 activities were evaluated to understand the mechanism of action induced by the anthocyanin extract, which revealed that the extract induced concentration-dependent increases in caspase 3, 8 and 9 activities in MCF 7 cell lines. Activation of the extrin-

Table 2. Activities of QR, GST, CYP 2 A1 in MCF 7 cells treated with different concentrations of the anthocyanin extract of *Cordyline australis*.

Concentration ($\mu\text{g/mL}$)	0	10	20	30	50
QR (U/mg protein)	1.01 ± 0.002	1.66 ± 0.03	2.89 ± 0.03	3.77 ± 0.06	4.4 ± 0.054
GST (U/mg protein)	0.54 ± 0.004	1.2 ± 0.089	2.12 ± 0.003	2.81 ± 0.005	3.0 ± 0.002
CYP2A1 (U/mg protein)	0.15 ± 0.001	0.20 ± 0.013	0.25 ± 0.007	0.287 ± 0.001	0.291 ± 0.01
GSH ($\mu\text{mol / g}$)	0.59 ± 0.004	1.8 ± 0.007	2.6 ± 0.043	3.5 ± 0.045	4.4 ± 0.005

sis apoptosis pathway was carried out through a ligand binding to a death receptor, which in turn performs recruitment, dimerization and activation of caspase 8 with the help of adapter proteins (FADD/TRADD). Apoptosis is initiated by activated caspase 8 either directly by cleaving and consequently activating executioner caspase (3, 6, and 7) or by activating the intrinsic apoptotic pathway through cleavage of BID to induce efficient cell death. Various cellular stresses can cause activation of the intrinsic mitochondrial apoptosis pathway, leading to the release of cytochrome c from the mitochondria and the formation of the apoptosome, comprised of APAF1, ATP, cytochrome c, and caspase 9, resulting in the activation of caspase-9, which in turn initiates apoptosis by cleaving and subsequently activating executioner caspases (McIlwain, 2013; Brentnall, 2013; Liu 2017).

Ediriweera *et al.* (2016) analyzed the anticancer activity of the bark hexane extract from *Mangifera zeylanica* in terms of cytotoxic and apoptosis and also its bioassay-guided fractionation to identify phytochemical constituents. The present cytotoxic data was more significant than the bark extract of *M. zeylanica*. Patil and Kim (2017) validated the efficacy of silver and gold nanoparticles in terms of antibacterial and anticancer activities. Deng *et al.* (2015) reviewed polyphenols of leaf and bark extracts of *Solidago canadensis* and its biological features. Elansary *et al.* (2020) compared antimicrobial, antioxidant and anticancer potentialities of *Malus baccata* var. *gracilis* and *M. toringoides* bark polyphenols. The viability data of the polyphenols was less than that of anthocyanin extract of *Cordyline australis*. Elansary *et al.*, (2020) profiled the polyphenols of *Quercus* spp with their pharmaceutical potentials. Cosarca *et al.* (2019) recorded aqueous extracts of spruce and beech bark as a source of polyphenols, tannins, antioxidants and correlated the components with the *in vitro* anti-tumor potential against two different cell lines. The present study of anthocyanins from *C. australis* profoundly substantiates the above research outputs.

Conclusion

Increasing *in vitro* experimental data from various research documents have proved that anthocyanins can interfere with multiple signal pathways to exert their antimetastatic activities. However, most of these experiments were performed under *in vitro* conditions. The present study also attempted the anticancer efficacy of anthocyanin extract from the Red star variety of *Cordyline australis*. The ethanolic extract showed 11 anthocyanin fractions (Delphinidin-3-o-galactoside, Delphinidin-3-o-glucoside, Cyanindin-3-o-glucoside, Cyanindin-3-o-rutinoside, Delphinidin-3-o-rutinoside, Peonidin-3, 5-di-o-glucoside, Petunidin-3-o-glucoside, Pelargonidin 3-o-glucoside, Peonidin-3-o-glucoside,

Peonidin-3-o-rutinoside & Malvidin-3-o-glucoside) and displayed its potentiality against MCF 7 cells in terms of inducing phase I and II enzymes, LDH leakage, caspase activates. Future studies should explore the connection between the intake of anthocyanin by the cells and its anticancer efficacy using *in vivo* animal models.

Conflict of interest

The authors declare that they have no conflict of interest.

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