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Phytochemical analysis and antioxidant defense of kiwifruit (*Actinidia deliciosa*) against pancreatic cancer and AAPH-induced RBCs hemolysis

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Abstract

Kiwifruit (*Actinidia deliciosa*) is a superb nutritional source of phenolic compounds that are beneficial in the prevention of different diseases. The main goal of the current study was to estimate the oxidative activity and compare the effect of the ethanol extract of this fruit's peel and flesh on pancreatic cancer against pancreatic cancer and in vitro 2, 2-azobis-2-amidinopropane dihydrochloride (AAPH) erythrocytes hemolytic oxidative damage. Firstly, HPLC was utilized to identify the phenolic compounds in each extract that showed that *p*-coumaric acid & ferulic acid (in the peel), syringic acid & cinnamic acid (in the flesh) were the most abundant. Concerning the anti-cancer effect, flesh extract exhibited more cytotoxic impact as detected by the reduction of PANC-1 cell line viability with IC_{50} of 232.89 µg/ml contrasted with the IC_{50} of the peel extract (556.60 µg/ml). Relative to ascorbic acid, both kiwi extracts have been considerably successful in diminishing hemolysis, thiobarbituric acid reactive substances (TBARS) and GSH levels of AAPH-induced RBCs. Interestingly, with 80 µg/ml, the ethanol peel extract returned the TBARS and GSH levels to the normal level of RBCs (0.022 and 2.86 nmole/mg hemoglobin, respectively).

Keywords: kiwifruit; polyphenol; anti-hemolytic; TBARS.

Practical Application: The oxidative activity of kiwifruit demonstrates that the flesh has an anti-pancreatic cancer effect while the peel can be utilized as an anti-hemolysis agent as compared with ascorbic acid.

1 Introduction

Many diseases, including Alzheimer, inflammation, atherosclerosis, and various kinds of malignant neoplasms, are attributed to macromolecular damage due to imbalanced radical scavenging and propagation of reactive oxygen species (ROS) (Fan et al., 2020; García-Sánchez et al., 2020). Elevated levels of ROS can oxidize biomolecules like DNA, proteins, and lipids leading to tissue damage, cell death, or degenerative processes (Brindisi et al., 2020; Zhang et al., 2020). It is imperative to evaluate and classify unprecedented natural sources of antioxidants for their pivotal function in the scavenging and repression of such radicals (Neha et al., 2019). Phytochemicals from natural sources such as fruits and vegetables have been documented as potential antioxidants (Sharifi-Rad et al., 2020) that are commonly administered in consequence of their vital action in both treatment and prevention of several diseases (Zhao et al., 2017).

Kiwifruit is an edible berry of many species of the *Actinidia* family. The 'Hayward' cultivar, which belongs to the *Actinidia deliciosa* genus, is the utmost common and commercially available green kiwifruit (Park et al., 2011). China is the major producer with an annual production of 2196727 tons in 2019; from the harvested area of 182566 ha. Also, New Zealand, Greece, and Italy are among the countries where it is cultivated (Food and Agriculture Organization of the United Nations, 2019).

A wide-ranging of phytochemicals as phenolic, flavonoids, vitamins, carbohydrates, folic acid, minerals, saponins, and tannins compounds have been recognized from kiwifruit extracts following extraction by solvents with various polarities (Bae et al., 2012; D'evoli et al., 2015; Wang et al., 2018; Alim et al., 2019). It contains abundant antioxidant compounds that facilitate its use in the treatment and prevention of a broad range of clinical conditions, including digestive disorders, rheumatism, dyspepsia, and cardiovascular diseases (Leontowicz et al., 2014; Podsedek et al., 2014; Peticila et al., 2015). The challenging anti-proliferative activity of kiwifruit towards human hepatocellular (HepG2), colon (HT-29), and gastric (GC) cell lines (Zuo et al., 2012; Lim et al., 2016) has already been demonstrated.

To our knowledge, no foregoing study has inspected the upshot of kiwifruit on pancreatic cancer cells or as an anti-hemolytic agent. Thus, the purpose of the current study is to examine and compare the phenolic and flavonoid content and the unique phenolic profile using HPLC of the ethanol extract of flesh and pericarp of *Actinidia deliciosa*. Comparing the biological functions of both extracts, such as pancreatic anti-cancer and anti-hemolytic action is the most significant objective.

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2 Materials and methods

2.1 Chemicals

Quercetin and nitro blue tetrazolium chloride were purchased from Cayman Chemical Co., USA. 3–4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Bio basic Inc., Canada while, RPMI-1640 medium was from Gibco; Thermo Fisher Scientific, Waltham, MA, USA, and fetal bovine serum (FBS) was obtained from Biological Industries, Cromwell, CT, USA. All other chemicals were of the analytical grades that were imported from Sigma-Aldrich Co., Germany.

2.2 Fruit extract preparation

Actinidia deliciosa (kiwifruit) was acquired from the local market in the season of July 2020. First, the flesh was separated from the skin, then homogenized by a high-speed hand blender. The ethanol extract was prepared by combining the flesh or peels separately with 70% ethanol (1:1 *w/v*) for 24 h at 30 °C. Subsequently, the filtrates were concentrated using a rotary vacuum evaporator (Buchi CH-9230, Germany) and thereafter lyophilized (Xiangyi, FD-10 Bench-top, China) and stored at –80 °C until use. The lyophilized powder was dissolved in ethanol 70% unless stated for the next experiments.

2.3 Total phenolic and flavonoid content estimation

Using the Folin-Ciocalteu reagent, total phenolic (TP) content was quantified as Singleton et al. (1999) method. By mixing 500 µl of fruit extract or methanol as a blank with 2500 µl of Folin-Ciocalteu reagent (10%) and 2500 µl of 7.5% $NaHCO_3$, the experiment tubes were incubated at 45 °C for 45 min. Total flavonoid (TF) was estimated by Didry et al. (1990) test, where 250 µl from each diluted extract was blended separately with (µl): 750 of 95% ethanol, 50 of 10% aluminium chloride, 50 of 1 M potassium acetate, and 1400 of distilled water, then incubated at 25 °C for 30 minutes. Finally, the absorbance of polyphenols and flavonoids mixture was read at 765 and 415 nm, respectively using UV-Spectrophotometer (Alpha-1502, USA). The amounts of TP and TF were valued as the equivalent gallic acid (GAE) and quercetin (QE)/g extract, respectively.

2.4 Phenolic compounds profile

The HPLC system of Singh et al. (2002) (pump PU-1580, UV detector UV-1570, injector equipped with a 20 µL loop) was used. The sample was separated using a 250 × 4.6-mm stainless-steel column discovery-C184 µm. The running conditions were maintained at a flow rate of 1 ml/min, and the temperature was at 25 °C. The solvent A was water containing 0.05% formic acid, and solvent B: acetonitrile/methanol (80:20, v/v). Each lyophilized kiwifruit extract sample (0.5 g) was extracted using ethanol (70%), then filtered and injected. The gradient conditions were as follows: 0–5 min, 10% B; 5–15 min, 10–18% B; 15–25 min, 18% B; 25–30 min, 18–25% B; 30–35 min, 25% B; 35–40 min, 25–35% B; 40–45 min, 35–60% B; 45–50 min 60–10% B; and 50–55 min with 10% B. The temperature of the column was controlled at 25 °C. Phenolic compounds existent in each extract have been defined by comparing the retention time (Rt) to the individual standards.

2.5 Anti-pancreatic cancer activity estimation

PANC-1 cell culture conditions

The human pancreatic cancer cell line PANC-1 (CRL-1469, isolated from a pancreatic carcinoma of ductal cell origin of a 56-year-old male) was purchased from American Tissue Cell Culture (ATCC, Manassas, VA, USA) then preserved in RPMI-1640 growth medium containing 2% fetal bovine serum (FBS). All media contained 100 units/ml penicillin and 100 µg/ml streptomycin. The cell line was held at 37 °C in a humidified incubator with CO_2 (5%). For the experiment, cells that had reached the exponential growth phase were chosen.

MTT assay

Rendering to the procedure of van de Loosdrecht et al. (1994), the anti-cancer activity was done using MTT assay. PANC-1 cells in the exponential growth phase were inoculated on 96-well plates at a density of 1 x10^5 cells/ml (100 µl per well), and sterile phosphate buffer saline (PBS) was added to the edge as well as a blank control. The plate was incubated at 37 °C with 5% CO_2 for one day to enable the cells' attachment. When the PANC-1 cells reached full confluence, the medium was withdrawn, and the monolayer cells were washed two times with 1 ml of trypsin (0.25%)/EDTA (0.05%) solution. The sample extract was first diluted in an RPMI medium (containing 2% serum), then 0.1 ml of each dilution was pipetted in each well, while the control well only contained the RPMI medium. At 37 °C, the incubated plates were examined for toxicity signs. Finally, 20 µl MTT (5 mg/ml PBS) was applied to each well, and then incubated for 1-5 h at 37 °C in 5% CO_2. To dissolve formazan (MTT metabolic product), two hundred µl of DMSO was poured into each well then stirred. Using a microplate reader (MR-96A, Mindray, China), the absorbance at 560 nm was recorded. The survival cell percentage was calculated using the next Equation 1:

$$\%\text{Cell viability} = \frac{\text{A treated cell}}{\text{A control cells}} \text{x}100 \quad (1)$$

2.6 Anti-hemolytic activity estimation

RBCs separation

From healthy Albino rats, the blood samples were obtained, then centrifuged (1000 x g) for ten minutes. After disposal of the supernatant, the pellet was re-washed and centrifuged 3-times with PBS (pH 7.4). Red blood cells (Erythrocytes, RBCs) were finally suspended in the same buffer to obtain approximately 50% hematocrit, stored at 4 °C and used within 6 h in the next experiments (Miki et al., 1987).

Measurement of RBCs hemolysis induced by AAPH

2, 2-azobis-2-amidinopropane dihydrochloride (AAPH) triggered erythrocyte hemolysis was quantified using the adapted method of Miki et al. (1987). Two hundred µl of each kiwi extract (100 to 500 µg/ml) were applied successively to 200 µl of AAPH (100 mM) with 200 µl of the previously obtained erythrocyte

suspension, then incubated with mild shaking at 37 °C for 3 h. Finally, the experiment tubes were diluted 20 times with PBS and centrifuged (at 1500 x g for 10 min) then A_{540} was recorded. The reference value was evaluated by mixing the hemolyzed erythrocytes with the hypotonic buffer (5 mM phosphate buffer pH 7.4; hemolysis of 100 percent). The next Equation 2 was applied to calculate the percentage of hemolysis:

$$\% \text{ Hemolysis} = \frac{\text{A sample supernatent}}{\text{Reference value}} \text{ x100} \quad (2)$$

Measurement of RBCs lipid peroxidation inhibition induced by AAPH

Erythrocytes were pre-incubated for a half-hour at 37 °C with varying concentrations of each kiwi extract, or ascorbic acid as a guide (20-100 µg/ml) or 0.2% ethanol as a negative control. Subsequently, 25 mM AAPH was applied to the erythrocytes suspension (5%) and incubated for 3 h at 37 °C with kind shaking. To interrupt the reaction, two ml of thiobarbituric acid reagent (0.375% TBA, 15% TCA in 0.2 M HCl) were used, after 1 h incubation in a boiling water bath. Finally, after cooling and centrifugation (at 1500 x g for 5 minutes), thiobarbituric acid reactive substances (TBARS) was spectrophotometrically monitored at wavelength 535 nm and valued per mg of hemoglobin (Borra et al., 2013).

Measurement of the reduced glutathione (GSH) concentration in hemolyzed RBCs

Using DTNB, known as Ellman's reagent, the GSH concentration was measured (Kunwar et al., 2007). Suspension of RBCs (5%) was subjected to hemolysis by 25 mM AAPH for 3 h, in the incidence of varying concentrations of each extract, precipitated by 10% TCA and then centrifuged (1500 x g) for 15 min. To 1000 µl of the supernatant, half ml of Ellman's reagent plus 3 ml of phosphate buffer (pH 8) was added. The yellow color established was read at wavelength 412 nm against the blank (distilled water) and GSH level was expressed as nmole DTNB/mg of hemoglobin.

2.7 Anti-oxidative activity estimation

Superoxide scavenging assay

It was determined in both extracts as the procedure of Nishikimi et al. (1972). One ml of each kiwi extract (in concentrations from 10 to 50 µg/ml) was blended with 1 ml from both nitro blue tetrazolium (156 µM) and NADH (468 µM) in PBS (0.1 M, pH 7.4) solutions. Reduced absorbance (A_{560}) indicated an increase in the scavenging percent as calculated by Equation 3.

$$\% \text{ Scavenging } = \frac{\text{A blank} - \text{ A sample}}{\text{A blank}} \text{ x100} \quad (3)$$

Hydroxyl scavenging assay

One ml of varying concentrations from each extract (20-100 µg/ml) was added to 2 ml of H_2O_2 solution (20 mM in PBS). After 10 min, the absorbance was estimated at 230 nm against a blank solution made up of H_2O_2. The percentage of scavenging was determined by Equation 3 (Ma et al., 2019).

2.8 Statistical analysis

The data were statistically analyzed by ANOVA test using SPSS version 20 at $p < 0.05$, then presented as the average of three replicates ± standard deviation (SD). The 50 percent inhibitory concentration (IC_{50}) was determined from the dose-response curve (Graph Pad Prism Version 6.0) achieved by plotting percent inhibition towards the different concentrations.

3 Results and discussion

3.1 Total phenolic (TP) and flavonoid (TF) content

The TP content, as determined by Folin–Ciocalteu reagent, was 14.2 and 10.2 mg GAE/g dry weight for the kiwi peel and flesh, respectively. The results verified that the phenolic content in the kiwi peel extract was 39.2% higher than flesh ethanol extract. TP content was estimated from the linear regression equation where $R^2 = 0.989$.

The most popular set of polyphenol substances in the human diet is flavonoids. Flavonoids have many biological activities such as antimutagenic, antiviral, and anti-oxidation (Alim et al., 2019). The TF content in kiwi peel (3.9 mg QE/g) was meaningfully higher ($p < 0.05$) than in flesh extract (1.6 mg QE/g), indicating that peel extract contained more than twice the flavonoid amount of the flesh. The TF was calculated by the equation of linear regression where $R^2 = 0.991$.

These findings are harmonious with other authors who found 16.67 ± 2.83 mg GAE/g and 12.95 ± 0.52 mg QE/g in the lyophilized aqueous kiwi extract (Bursal & Gülçin, 2011), and 9.60 mg GAE/g dw & 92.1 µg catechin (CE)/g dw in methanol extract of *A. deliciosa* pulp (Park et al., 2011). Alim et al. (2019) and Wang et al. (2018) have also ensured our results that the peel extract has an elevated degree of phenolic and flavonoids content in contrast with the flesh extract, 28.79 vs. 15.44 mg GAE/g and 13.96 vs. 2.1 mg rutin/g dw, respectively.

3.2 HPLC analysis

To recognize the exact polyphenols in each lyophilized kiwifruit ethanol extract, the polyphenol composition was explored as appeared in Figure 1 and concentrations thereof in Table 1. Ten phenolic compound standards have been detected, including five hydroxycinnamic acids (ferulic, caffeic, chlorogenic, cinnamic and *p*-coumaric acids), two hydroxybenzoic acids (syringic acid and ellagic acid), one flavonol (quercetin), and two flavan-3-ols ((+)-catechin and it's derivative: catechol). The overall content of polyphenols in the ethanol extract of kiwi peel surpasses the flesh content, which is consistent with other studies (Bursal & Gülçin, 2011; Wang et al., 2018). Results indicated that syringic acid (11.25 mg/kg) was the key phenolic compound in kiwi pulp extract. It was accompanied by cinnamic acid (10.25 mg/kg) and ellagic acid (7.14 mg/kg) with low levels of catechin and quercetin. On the opposite

hand, the most plentiful phenolic compounds in kiwi peel extract were hydroxycinnamic acids *i.e. p*-coumaric acid and ferulic acid (15.5 and 10.3 mg/kg, correspondingly) that are rarely enclosed in kiwi flesh. Other researchers figured that the dominant components of phenolics in the kiwi flesh were (+)-catechin, chlorogenic acid, rutin, (-)-epicatechin and quercetin (Kim et al., 2009). Rutin and quercetin were detected by Wang et al. (2018) in both the flesh and peel extract of *A. deliciosa* cv. Hawyard, but the chief phenolic compound was L-epicatechin. The divergences between our findings may be attributable to the diverse kiwifruit cultivars, cultivation conditions and the solvent used.

3.3 Anti-pancreatic cancer effect

Due to its rapid growth, early metastases, and poor response to any known therapies, pancreatic cancer stands as one of the troublesome types of cancer. As the fourth reason for cancer mortality and with anticipation of being the second cause after lung cancer in 2030, there is an immediate need to discover natural and healthy food to prevent such disease (Aizikovich, 2020). Figure 2 demonstrates the cytotoxic impact of the ethanol extract of kiwi flesh and peels on PANC-1 cells viability as estimated by the MTT assay. Both extracts have been able to decrease the viability rate of the tumor cells in a dosage-dependent manner. The flesh has potent cytotoxicity compared to kiwi peels. More than 90% of PANC-1 cells died at 1000 µg flesh extract/ml. By increasing the concentration of the peel extract from 125 to 1000 µg/ml, the growth rate of cells decreased from 99.71 to 22.22%. The same illustration also demonstrated the dosage needed for 50% inhibition (IC_{50}) of 232.89 µg/ml for kiwi flesh and 556.6 µg/ml for peel extract. The morphological specification of the treated cell line, as opposed to the untreated cells, is shown in Figure 3. The cells treated with each extract

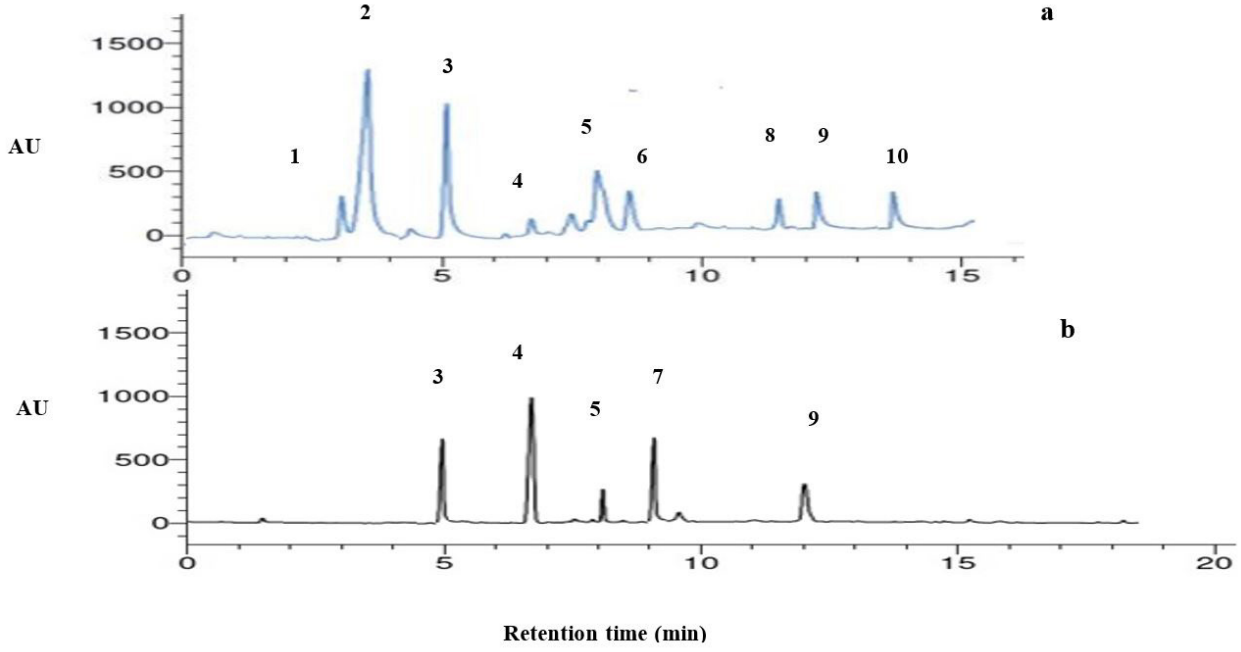

Figure 1. HPLC chromatograms of the free phenolic compounds of the kiwifruit ethanolic extracts. (a) Flesh extract; (b) Peel extract. 1: Catechin, 2: Syringic acid, 3: Cinnamic acid, 4: *p*-Coumaric acid, 5: Ellagic acid, 6: Caffeic acid, 7: Ferulic acid, 8: Catechol, 9: Chlorogenic acid, 10: Quercetin.

Table 1. The Content of phenolic compounds in kiwifruit ethanol extracts (µg/g DW) as detected by HPLC (mean, n = 3).

No.	Retention time (min)	Phenolic compound	Molecular weight	Flesh extract	Peel extract
1	2.99	Catechin	290.26	1.20	-
2	3.30	Syringic acid	198.17	11.25	-
3	5.10	Cinnamic acid	148.16	10.25	9.25
4	6.99	*p*-coumaric acid	164.04	0.73	15.50
5	8.01	Ellagic acid	302.19	7.14	4.02
6	9.12	Caffeic acid	180.16	6.55	-
7	9.00	Ferulic acid	194.18	-	10.30
8	11.20	Catechol	110.11	1.22	-
9	12.10	Chlorogenic acid	354.31	2.31	6.29
10	14.00	Quercetin	302.23	2.55	-
		Total		42.00	45.36

exhibited morphological cell death characteristics such as wrinkles, rounding and the resignation of the cells, which increased by increasing the extract concentrations.

Our results established that the anti-pancreatic adenocarcinoma activity of kiwi flesh was more potent than that of peels. This was strongly associated with its phenolic profile and the compounds found, in particular quercetin, caffeic acid and syringic acid, which mainly exist in the extract of the flesh and not found in the peel. Quercetin is a pleiotropic kinase inhibitor that has demonstrated its anti-pancreatic effect (Russo et al., 2014), while caffeic and syringic acids have an inhibitory effect on different cancer cells proliferation (Rajendra Prasad et al., 2011). This finding is controversial with previous results of Alim et al. (2019) where ethanol extract of kiwi (*A. Chinensis*) flesh and peel exhibited HepG2 anti-proliferation with IC_{50} of 291 and 170 µg/ml, respectively. In contrast, acetone extract of kiwi peels (*A. deliciosa*) did not have any effect on breast MCF-7 and hepatocellular carcinoma HepG2 cell lines (Salama et al., 2018). The methanol extract *A. aruguta* pulp showed an anti-proliferative effect on Hep3B and Hela (IC_{50} of 0.3 and 0.2 µg/ml) but not on LoVo, HepG2, HT29 cell lines (Lim et al., 2016). Our study covers the aspects of new evidence for the upshot of kiwifruit on pancreatic cancer that has not been previously demonstrated.

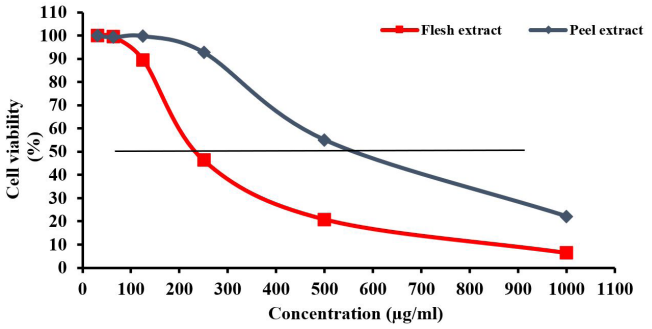

Figure 2. Effect of ethanol extracts of kiwifruit on the PANC-1 cell line viability.

3.4 Anti-hemolytic effect

Inhibition of RBCs hemolysis

Erythrocytes hemolysis is considered a perfect model for stating the free radical intermediate oxidative damage to the cell membrane and for evaluating the anti-oxidation action (Qin et al., 2020). AAPH has been proven to influence hemolysis by raising intracellular free radicals. The proteins and lipids of the erythrocyte membrane are eventually attacked by ROS or free radicals, which are very responsive in nature, causing irreversible damage. Typically, this process ends with erythrocyte hemolysis or the degradation of healthy erythrocytes (Yang et al., 2017; Rashidpoura et al., 2021). The scavenging of peroxyl radicals was therefore investigated using different concentrations of kiwi flesh and peel extracts and standard ascorbic acid to estimate the activity of kiwi fruit against AAPH-induced hemolysis of RBCs. The study demonstrated that the hemolysis percentage diminished in a concentration-dependent way (Figure 4) when the ethanol extract of kiwifruit (between 100 and 500 µg/ml) was applied. At 100 µg/ml extract concentration, when kiwi flesh, peel, and ascorbic acid were added, the percentage of RBCs hemolysis was 88.23%, 78.71% and 62.49%, correspondingly. Maximum inhibition of RBCs hemolysis was recorded, at a concentration of 500 µg/ml, by ascorbic acid (25.07%) afterwards, the peels extract (42.92%) then by the flesh extract (58.19%). Generally, the kiwi peel extract demonstrated a significantly higher value compared to the kiwi flesh extract ($p < 0.05$). In addition, either kiwi flesh or peel extract exhibited slightly lesser values ($p < 0.05$) than their reference. The IC_{50} was found to be 597.94 ± 2.3 µg/ml for pulp, 387.37 ± 2.1 µg/ml for peel, and 201.36 ± 2.9 µg/ml for

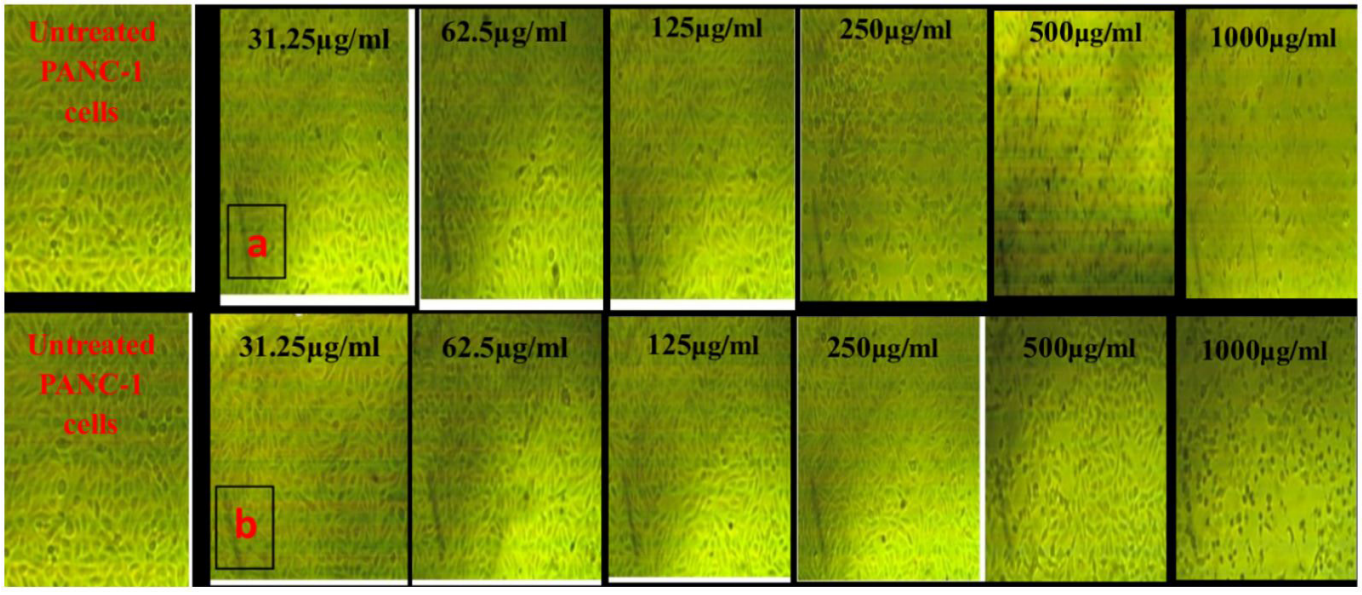

Figure 3. Comparison of cytotoxicity effect of (a) kiwi flesh and (b) kiwi peel extracts at different concentrations on PANC-1 cells compared with untreated cells.

the standard ascorbic acid. Kiwi peel is moderately effective in preventing progressive hemolysis of RBCs. It may be related to the identified antioxidant phenolic compound, ellagic acid, which is naturally synthesized in plants as a protectant against infections and pests (Vattem & Shetty, 2005). This study highlighted the positive cellular anti-oxidative abilities of kiwifruit for the first time, which had previously been documented by other plants extracts as mulberry leaves (Choi et al., 2013), pomelo fruit (Wang et al., 2019), and osthole as an active component of *Cnidium monnieri* (Rashidpoura et al., 2021).

Inhibition of RBCs lipid peroxidation

Just as the membrane of erythrocytes is rich in polyunsaturated fatty acids, AAPH-initiated radicals can oxidize membrane lipids at physiological temperature, causing rapid membrane injury and destruction of integrity, prompting lipid peroxidation (Yang et al., 2017; Rashidpoura et al., 2021). In this examination, the lipid peroxidation rate of RBCs was estimated by calculating TBARS released. Figure 5 shows the varying amounts of TBARS in RBCs (after AAPH-induced harm) without or with various concentrations of kiwi flesh and peel extracts, contrasted with ascorbic acid. The standard baseline level of TBARS was established to be 0.024 ± 0.03 nmole/mg hemoglobin in RBCs (without AAPH); and when incubated with AAPH, the TBARS level increased to 0.092 ± 0.07 nmole/mg hemoglobin. In the case of kiwifruit flesh and peel extracts, or ascorbic acid, there was indeed a steady decline in the TBARS level in AAPH-induced RBCs, and this inhibition increased with increasing the concentrations tested. For example, at a concentration of 20 µg/ml, TBARS formation was significantly diminished ($p < 0.05$) by ascorbic acid and kiwi flesh and peel extracts starting from 0.092 to 0.063, 0.059, and 0.052 nmole of TBARS/mg hemoglobin, respectively. In general, all kiwi extracts have been substantially more effective in reducing TBARS levels of RBCs than ascorbic acid. Overall, the lipid peroxidation inhibition by kiwi peel extract at all concentrations was visibly higher than the flesh ($p < 0.05$). Obviously, peel extract returned the TBARS level at 80 and 100 µg/ml concentrations to the normal basal level of natural RBCs (0.022 nmole/mg hemoglobin). Since kiwifruit is rich in ascorbic acid and polyphenols, we assume that these components may have contributed to inhibiting AAPH-induced free radical development and preventing free radical-induced oxidative hemolysis. No published work has inspected the impact of kiwifruit on RBCs hemolysis. Further research is needed to distinguish and extract the particular compounds responsible for this phenomenon from kiwifruit.

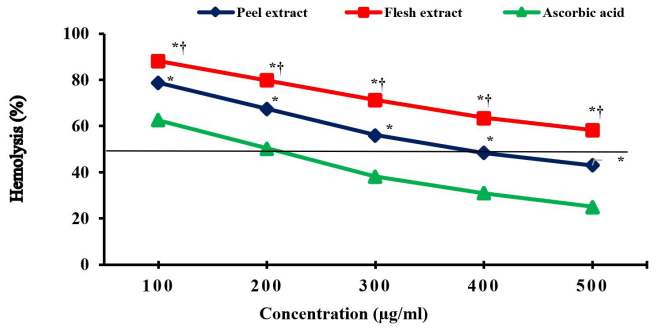

Figure 4. Effect of kiwi peel and flesh extracts against AAPH-induced hemolysis compared with ascorbic acid. *Results of flesh or peel extract are significant at p < 0.05 compared with ascorbic acid. † Results of flesh extract are significant at p < 0.05 compared with the peel extract.

The reduced glutathione (GSH) concentration in hemolyzed RBCs

The most plenteous thiol segment found in mammalian cells is GSH, which has a diversity of pivotal cell functions as keeping up the essential thiol status of proteins by preventing oxidation of sulfahydryl groups, dipping disulphide bonds induced by oxidative stress, or by free radicals scavenging. Through oxidative stress, the cellular pool of GSH has declined (Zhang et al., 2014). Thus, cellular GSH depletion is well-known to be a foot marker of oxidative stress and correlated with increased lipid peroxidation and protein oxidation (Yang et al., 2017; Rashidpoura et al., 2021). Figure 6 shows the changes in GSH levels of RBCs following AAPH induction with the presence of different kiwi flesh and peel extracts concentrations (20-100 µg/ml), compared to ascorbic acid. The normal basal GSH level in RBCs (control) was found to be 2.86 ± 0.19 nmole/mg hemoglobin. Incubation of erythrocytes with AAPH (25 mM) for 3 h caused a greater diminution (≈57%) in the glutathione level from the basal to 1.23 ± 0.27 nmole/mg hemoglobin. The dramatic drop in GSH levels suggests that exposure to AAPH is severe oxidative stress. In the existence of kiwi flesh and peel extracts or ascorbic acid, a gradual upsurge in GSH levels with increasing concentrations was observed. Results also display that all the tested concentrations of kiwi extracts or ascorbic acid, significantly increased GSH level in RBCs treated with AAPH. Relative to ascorbic acid, kiwi peels and flesh extracts have been found to significantly improve ($p < 0.05$) the GSH level. At two concentrations (80 and 100 µg/ml), the GSH of RBCs treated with kiwi peel extract reached the control level (2.86±0.19 nmole/mg hemoglobin) with no significant variations. The observed decrease in GSH levels in kiwifruit extracts AAPH-pretreated RBCs samples may be due to an excess accumulation of phenoxyl radicals, which oxidizes cellular GSH to GSSG (Choi et al., 2013). The elevation of erythrocyte GSH levels by kiwifruit extracts; supports the antioxidant properties of this fruit, which may point to lower levels of TBARS. It mainly correlated with the protective power of the identified polyphenols, especially ellagic acid, caffeic acid, syringic acid and quercetin (Choi et al., 2013). Once more, we have given proof that kiwifruit has an outstanding antioxidant activity against AAPH-induced GSH depletion in RBCs higher than pure ascorbic acid. However, their mechanism of action in cell signaling related molecules warrants future investigation.

3.5 Anti-oxidative activity

It was critical to examine the anti-oxidative efficiency of the extracts prepared in order to attribute the critical role of natural antioxidants in the scavenging and inhibition of free radicals demonstrated in the previous experiments. Consequently, besides the estimation of individual bioactive compounds (e.g. polyphenols and flavonoids) in each extract, two not common complemented antioxidant activity assays have been determined. HO• and superoxide anion are the utmost active anions among

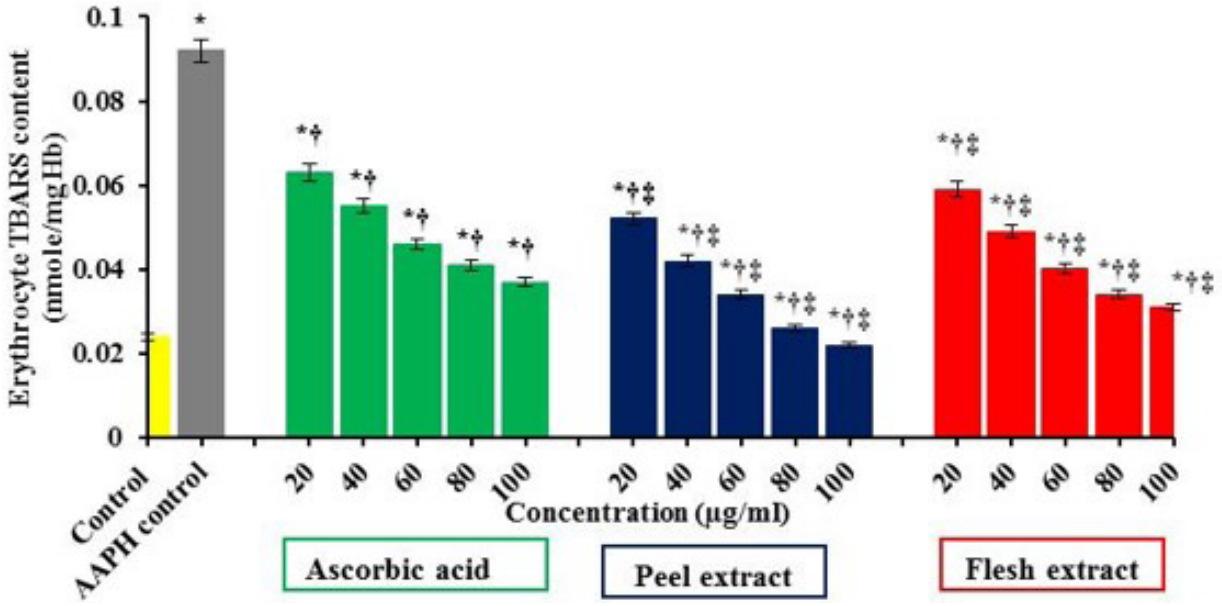

Figure 5. The TBARS levels of AAPH-induced erythrocytes restored by the kiwi peel and flesh extracts. Erythrocyte suspension at 5% hematocrit was incubated with PBS (control) or pre-incubated with different concentrations of peel, flesh or ascorbic acid (20-100 µg/ml) for 30 min. Then, the erythrocytes were incubated with AAPH (25mM) for 3 h. Erythrocyte TBARS content was expressed as nmole/mg Hb. Values are expressed as Mean (n = 3) ± SD. *: Results are significant at $p < 0.05$ compared with untreated control cells. †: Results are significant at $p<0.05$ compared with cells treated with AAPH alone. ‡: Results of flesh extract or peel extract are significant at $p < 0.05$ compared with the same concentration of ascorbic acid. AAPH: 2, 2′-azobis (2-amidinopropane) dihydrochloride; PBS: Phosphate buffer saline; SD: Standard deviation.

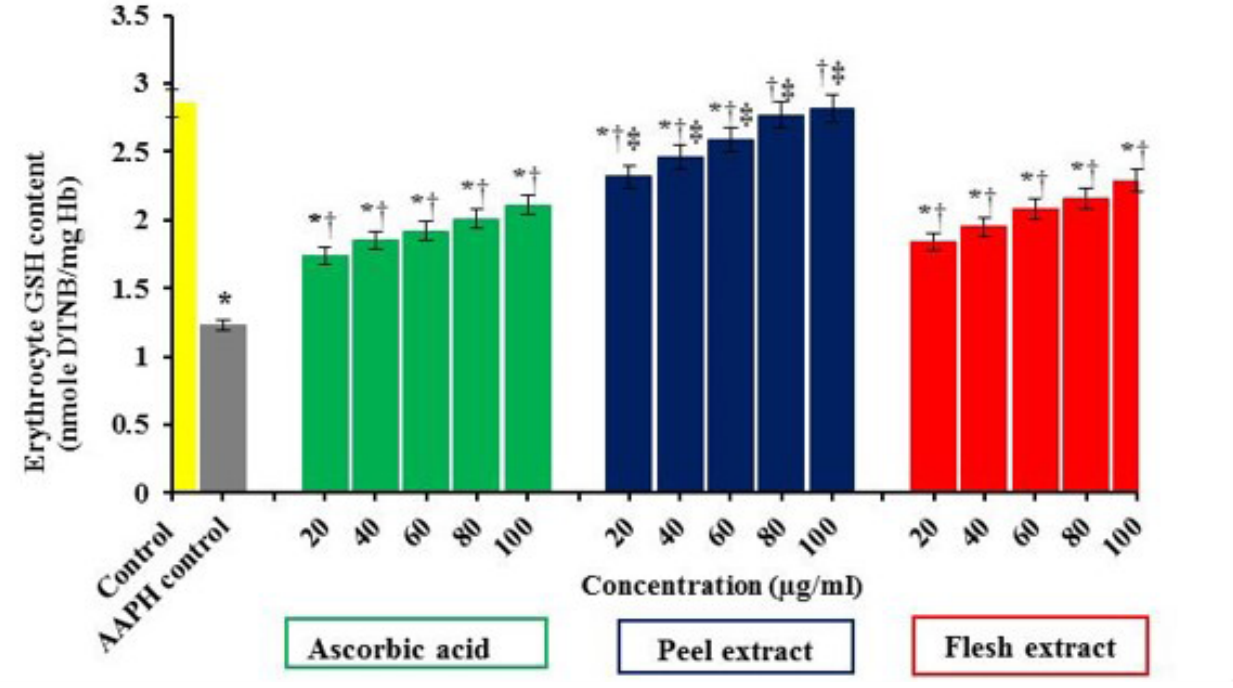

Figure 6. The GSH levels of AAPH-induced erythrocytes restored by the kiwi peel and flesh extracts. Erythrocyte suspension at 5% hematocrit was incubated with PBS (control) or pre-incubated with different concentrations of peel, flesh or ascorbic acid (20-100 µg/ml) for 30 min. Then erythrocytes were incubated with AAPH (25mM) for 3 h. Erythrocyte GSH content was expressed as nmole/mg Hb. Values are expressed as Mean (n = 3) ± SD. *: Results are significant at $p < 0.05$ compared with untreated control cells. †: Results are significant at $p < 0.05$ compared with cells treated with AAPH alone. ‡: Results of flesh extract or peel extract are significant at $p < 0.05$ compared with the same concentration of ascorbic acid. AAPH: 2, 2′-azobis (2-amidinopropane) dihydrochloride; DTNB: 5, 5-dithiobis-2-nitrobenzoic acid; GSH: Glutathione; PBS: Phosphate buffer saline; SD: Standard deviation.

a range of oxygen radicals that can trigger oxidative damage to numerous biomolecules causing ageing, cancer and numerous diseases (Dastmalchi et al., 2020).

Superoxide anion scavenging activity

The level of superoxide inhibition action of flesh, skin, in contrast with ascorbic acid (Figure 7a) ranged from 31.1 to 60.3%, 50.8 to 79.1% and 57.4 to 87.7%, respectively. Concerning IC_{50}, it was 27.00, 9.84, and 8.71 µg/ml for flesh, peel and ascorbic acid, respectively. These results revealed that the extract of the skin part exhibits the approximately triple strength of the flesh part towards superoxide anion ($p < 0.05$). Indeed, referring to the standard compound, IC_{50} of kiwi peel is nearly the ascorbic acid IC_{50} with no considerable difference. This may be linked to the presence of phenolic compounds in particular p-coumaric, ferulic, which have not been detected in the flesh extract (Table 1). According to Dastmalchi et al. (2020) explanation, phenolic compounds react with free radicals, give them electrons, and facilitate the conversion of free radicals to a stable formula.

Hydroxyl radical scavenging activity

Figure 7b clarified the dose-dependent curve of the $HO^{\bullet}$ scavenging activities of kiwi flesh and peel extracts separately relative to ascorbic acid. H_2O_2 scavenging power of the flesh, peel, and ascorbic acid increased from 66.6 to 80.9%, 77.9 to 90.4% and 84.4 to 91.5%, respectively by elevating the extract concentration from 20 to 40 µg/ml. The increment in concentration of the extract slightly, without significant differences, raises the free hydroxyl radical scavenging percentage to the maximum at 100 µg/ml. Ascorbic acid had a relatively greater activity related to the flesh. Starting from the concentration of 80 µg/ml, the kiwi peel's H_2O_2 scavenging was marginally higher than that

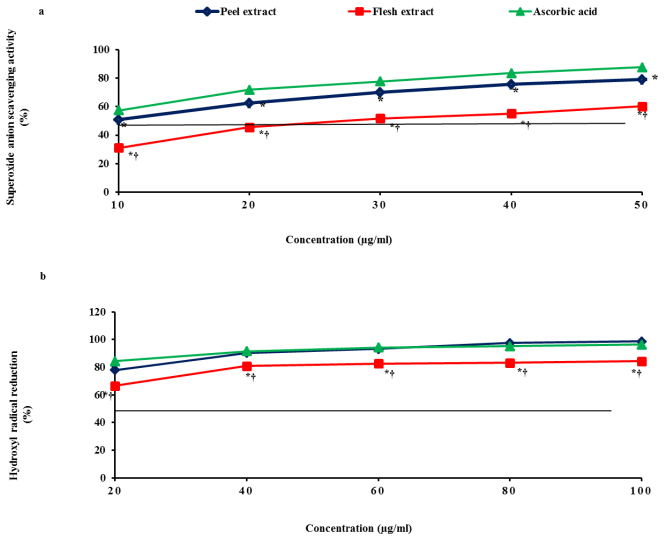

Figure 7. (a) Superoxide anion scavenging activity; (b) Hydroxyl radical reduction activity of kiwi peel and flesh extract comparing with ascorbic acid. *Results of flesh or peel extract are significant at $p < 0.05$ compared with ascorbic acid. † Results of flesh extract are significant at $p < 0.05$ compared with the peel extract.

of standard ascorbic acid. The least IC_{50} value was disclosed by ascorbic acid (11.84 µg/ml) followed by peel extract (12.83 µg/ml) then flesh extract (15.01 µg/ml). Relative to the extract of peel and pure ascorbic acid, the flesh ethanol extract separately showed a considerable ($p < 0.05$) higher IC_{50} (16.99% and 26.77%, correspondingly). Again, no substantial difference was noticed between IC_{50} of kiwi's peel extract and vitamin C (standard) which confirmed the potent scavenging effect of these fruit peels. A previous study done by Bursal & Gülçin (2011) also reported the antioxidant and antiradical activities of *Actinidia deliciosa* against superoxide anion and hydroxyl radical compared to the standard antioxidant compounds.

4 Conclusion

In conclusion, the results of the current study established the cytotoxicity of the kiwi fruit flesh extract against PANC-1 cell line. Also, attributable to its content of antioxidants, the peel extract of this fruit showed an effective anti-hemolytic activity against AAPH-prompted hemolysis and lipid peroxidation. Additional research is required to prove the anticancer mechanism of the kiwifruit parts in animal models, to understand the therapeutic power of this fruit on pancreatic cancer and the potential benefits for clinical practice in the future.

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