1.3.5. To determine the effect of kaffir lime peel ethanolic extract with different amount on cell permeability of Caco-2 cells under $H_2O_2$-induced oxidative stress by measuring transepithelial electrical resistance (TEER) value and evaluating FITC-dextran permeability.

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PC12 cells, where hesperetin, hesperidin, and neohesperidin could significantly enhance the cell viability and reduced the intracellular ROS production under $H_2O_2$-induced oxidative stress (Hwang & Yen, 2008). In human hepatocytes, hesperidin was able to prevent the tertiary butyl hydrogen peroxide (TBHP)-induced cell damage by upregulating heme oxygenase-1 (HO-1), a cytoprotective enzyme, resulting in an increased cell viability and a decreased intracellular ROS production (M. Chen et al., 2010). In another study, L6 myoblast cell under TBHP-induced stress was pre-treated with naringin leading to a decrement of intracellular ROS production (Dhanya et al., 2015). It was revealed that glutathione (GSH), an endogenous antioxidant, was restored to the control group levels. Diosmin exhibited an ameliorating potential in endothelial cells under $H_2O_2$-induced oxidative stress (Wójciak et al., 2022). Cell viability was improved and intracellular ROS production was prevented. Diosmin could recover the activity of cellular antioxidant enzymes. Deguelin, a newly identified flavonoid compound in *C. hystrix* peel ethanolic extract based on our study, was reported to attenuated oxidative stress in diabetic rats (J. Chen et al., 2018).

## 2.5. Conclusions

In conclusion, we evaluated the antioxidant potential of *C. hystrix* peel ethanolic extract under $H_2O_2$-induced oxidative stress in Caco-2 cells, for the first time. The proven capability to reduce oxidative stress was supported by the flavonoids contained in the sample and the antioxidant activities exhibited by the sample. CHPPE contained potential natural antioxidant compounds, including hesperidin, diosmin, neohesperidin, naringin, and deguelin. *In vitro* antioxidative capacities of CHPPE were expressed through DPPH radical scavenging, FRAP, and ORAC assays. Our results also demonstrated the functional properties of *C. hystrix* peel ethanolic extract in a cellular model, showing the ability to significantly improve the cell viability and reduce the presence of intracellular ROS. This implies that *C. hystrix* peel ethanolic extract can be utilized as a functional ingredient in food and pharmaceutical industries to promote human health against oxidative stress.

## 2.6. References

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replications (n=2), except the red bar (100 µM $H_2O_2$) and green bar (1 µg/mL CHPPE), n=1.

### 3.5. Conclusions

In conclusion, oxidative stress induced by $H_2O_2$ decreased TEER value of Caco-2 cells and increased FITC-dextran permeability, indicating intestinal barrier dysfunction. CHPPE had the potential to attenuate oxidative stress due to containing phenolic compounds. However, significant results could not be achieved by present study. Further research is highly needed.

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