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High-value biological compounds in jackfruit (*Artocarpus heterophyllus* Lam.): Postulates of their anticancer possible mechanisms.

Compuestos de alto valor biológico en jaca (*Artocarpus heterophyllus* Lam.): Postulados de sus posibles mecanismos anticancerígenos.

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ABSTRACT

Jackfruit contains high-value biological compounds with important biological activity in the inhibition of carcinogenesis. This study aimed to identify the metabolites in a methanolic fraction of an extract of jackfruit; which was reported with antimutagenic and antiproliferative activity but not characterized. Identification of compounds and reports of their documented anticancer activity permitted the establishment of postulates about their possible inhibition mechanisms of the carcinogenesis process. The profile includes 21 compounds, among them apigenin, artocarpin, cudraflavone C, moracin C, and *β*-carotene are the metabolites with important biological activity against cancer cells in the different phases of carcinogenesis.

KEY WORDS: Jackfruit, anticancer metabolites, biological activity, carcinogenesis process, apoptosis.

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Revista Bio ciencias
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RESUMEN

La jaca contiene compuestos de alto valor biológico con importante actividad biológica en la inhibición de la carcinogénesis. Este estudio tuvo como objetivo identificar los metabolitos en una fracción metanólica de un extracto de jaca; la cual fue reportada con actividad antimutagénica y antiproliferativa, pero no caracterizada. La identificación de los compuestos y los informes de su actividad anticancerígena documentada, permitieron establecer postulados sobre sus posibles mecanismos de inhibición del proceso de carcinogénesis. El perfil incluye 21 compuestos, entre ellos, apigenina, artocarpina, cudraflavona C, moracina C y *β*-caroteno son los metabolitos con importante actividad biológica contra las células cancerosas en las diferentes fases de la carcinogénesis.

PALABRAS CLAVE: Jaca, metabolitos anticancerígenos, actividad biológica, proceso de carcinogénesis, apoptósis.

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is a huge fruit, inside the fruit, it contains large edible bulbs, which are characterized by being fleshy, yellow, and fibrous (Zhang *et al*., 2018). According to Ruiz-Montañez *et al*. (2015) jackfruit contains a significant amount of secondary metabolites which are classified as high-value biological compounds (HVBC). These HVBCs have antioxidant, antimutagenic, and antiproliferative properties. The authors reported that the compounds present in the methanolic fraction of the jackfruit extract show a greater inhibition response during the development of carcinogenesis (Ruiz-Montañez *et al*., 2015). However, the identification of compounds that possess these biological activities has not been reported. The process of carcinogenesis may be divided into at least three stages: initiation, promotion, and progression. The last one is exclusive of the malignant transformation and implies the ability to invade nearby or distant tissues. For the metastatic process (proliferation of cancer to other tissues) a series of mechanisms are required, such as angiogenesis (creation of new blood vessels to feed cancer), matrix degradation, cell migration, and evasion of host immune response, as well as metastatic colonization. This last is considered the most serious phase in cancer progression since cancer cells have invaded other body tissues (Witsch *et al*., 2010). Currently, a lot of cancer research has been developed *in vitro* assays, and the explanation of metabolites' mechanisms of action has been elucidated in different biological pathways. In this sense is important to know in a feasible form the multiple possible vias that anticancer metabolites in jackfruit can act against cancer cells. Postulates based on cancer studies could be a comprehensible manner to explain the benefits of these compounds. Thus, focusing on the importance to know the identity of compounds responsible for the effects against cancer cells, the objective of this study was to

Revista Bio ciencias
ISSN 2007-3380

identify the phytocompounds present in the jackfruit fruit extract and develop postulates regarding the inhibition mechanisms of carcinogenesis reported in cancer research.

Material and Methods

Raw material and chemicals

Jackfruit fruits were provided by Frutos Tropicales de la Bahia, S.P.R. of R.L. in Compostela, Nayarit, Mexico, at the physiological maturity stage. The fruits were kept at room temperature (28 ± 3 °C, 70-75 % RH) for 5-6 days until ripe. The chemicals *n*-hexane (65 % purity), methanol (99.80 % purity), and glacial acetic acid (99.7 % purity) were purchased from Jalmek (San Nicolás de los Garza, Nuevo León, Mexico). HPLC-grade acetonitrile was obtained from TEVIA (Fairfield, Ohio, USA). Authentical standards of Table 1 were purchased from Sigma-Aldrich (St. Louis, MO, USA) and BioCrick (Chengdu, China).

Ultrasound-assisted extraction of HVBC

A sample of 50 g of jackfruit pulp lyophilized at -50 °C and 0.12 mbar (Labconco FreeZone Freeze-Dry, model 4.5, Kansas, MO, USA) was mixed with *n*-hexane in a 1:10 ratio (g of sample:mL of solvent). The mixture was placed in an ultrasonic bath (CD-4820, Guangdong, China) for 30 min at 42 kHz. Subsequently, the mixture was filtered and concentrated in a rotary evaporator (IKA, RV10, USA) under reduced pressure (-90 kPa) at 200 rpm and 40 °C, until solvent-free extract. Partition of the extract was carried out by dissolving it in a methanol-hexane mixture (2:3 v/v). The immiscible phases were separated in a separatory funnel for 3 h at 4 °C. The methanolic partition was concentrated under reduced pressure and further dried with a gentle N_2 stream at 500 mg, and finally was stored in an amber vial at -20 °C until analysis (Ruiz-Montañez *et al*., 2015).

HPLC-MS analysis of HVBC

The identification of HVBC was performed by using high-performance liquid chromatography coupled to mass spectrometry with a 1260 Infinity HPLC system (Agilent, Santa Clara, USA) equipped with a quaternary pump coupled to a 6120 quadrupole mass detector (Agilent, Santa Clara, USA). A Poroshell 120 EC-C18 column (2.7 µm, 4.6 × 50 mm) thermostated at 25 °C was employed for separation. A sample of 5 mg was diluted in 5 mL of methanol and filtered, and 5 µL were injected and eluted at a flow rate of 0.1 mL/min. A solution of acetonitrile (solvent A) and acidified water with 0.2 % glacial acetic acid (solvent B) was used as mobile phase in a gradient of 90/10 (vA/vB). The mass spectra were performed with N_2 as a gas pressure nebulizer (2 psi), the dry gas flow rate was 9 L/min, and the solvation temperature of 300 °C. The capillary voltage was adjusted to 3000 V in negative scanning mode. For identification, the injection of authentical standards was made under the same chromatographic conditions, for tentative identification, the molecular mass acquired was compared with reports in the bibliography; as well as a comparison of the mass spectral obtained was made with NIST and mass-spectra MassBank databases. The samples were analyzed in triplicate.

Revista Bio ciencias
ISSN 2007-3380

Results and Discussion

Identification of HVBC in the methanolic extract of jackfruit

The HVBC in the methanolic fraction of jackfruit extract were identified and those metabolites reported with anticancer activity were chosen to develop postulates about their possible mechanisms of action against cancer cells. Different compounds were identified, such as cudraflavone C, artocarpin, apigenin, moracin C and *β*-carotene (Table 1).

Table 1. Chromatographic and spectral data of compounds identified in the methanolic extract of jackfruit

Compounds	Molecular mass (*m/z*)	Retention time (min)
Organic acids		
Quinic acid[a]	192.06	4.243
Malic acid[a]	134.02	4.645
Citric acid[a]	192.02	5.536
Phenolic acids		
2,4 Dihydroxybenzoic acid methyl ester[c]	168.14	1.071
Chlorogenic acid[a]	354.31	1.083
Caffeic acid[a]	180.16	4.661
Gallic acid[a]	171.00	5.096
Flavonoids		
Cyanomaclurin	288.25	0.825
Albanine[c]	354.35	2.021
Cudraflavone C[b,c,d]	422.47	5.042
Artoheterophilin B	504.06	5.069
Brosimone I	420.61	5.070
Norarthocarpine	422.47	5.101
Artocarpin[a,b,c,e]	436.50	5.143
Gemichalcone	516.54	5.186
Morachalcone A	340.11	5.252
Apigenin[a,b,c]	270.00	5.290
Moracin C[a,b,c]	308.33	5.520
Catechin[a]	290.07	6.120
Carotenoids		
β-carotene[a,f]	536.00	6.440
Crocetin[a]	328.40	9.509

[a]Compounds identified by using authentical standards. Other compounds were tentatively identified based on their MS data spectra, data from the literature, and by comparison of the mass spectra obtained with those of databases NIST and MassBank (https://massbank.eu/MassBank/Search).

Compounds previously reported in jackfruit: [b]Fang *et al*. (2008), [c]Zheng *et al*. (2014), [d]Yao *et al*. (2016), [e]Sun *et al*. (2017), and [f]Ranasinghe *et al.* (2019).

Revista Bio ciencias
ISSN 2007-3380

Postulates of the mechanisms for inhibition of carcinogenesis by HVBC present in jackfruit extract

According to the HVBC identified and the reported studies on the mechanisms for inhibition in different phases of the cancer process, the following postulates are proposed.

Apigenin has antimutagenic and antiproliferative properties against cancer cells

Apigenin is a dietary flavonoid present in several plants. Important anti-inflammatory, antioxidant, antibacterial, and antiviral activities have been attributed to this compound. Currently, apigenin has been extensively investigated for its anticancer activity and low toxicity. *In vitro* and *in vivo* studies have shown its capacity of suppressing human cancers by triggering apoptosis and autophagy. Mechanisms involve cell cycle arrest, suppressing cell migration and invasion, and promoting an immune response (Yan *et al*., 2017). In this sense, Shukla *et al*. (2014) reported that apigenin treatment of androgen-resistant human prostate cancer cell lines PC-3 and DU145 resulted in apoptosis and reduced the cell viability caused by a decrease in Bcl-2 and Bcl-xL. Additionally, an increase in Bax protein concentration, accompanied by dose-dependent suppression of XIAP proteins, c-IAP1, c-IAP2, and survivin, was observed.

Regarding autophagy, studies carried out by Tong *et al*. (2012) reported that apigenin-triggered autophagy induces the activation of AMPK-activated protein kinase and the mTOR pathway in human keratinocytes. In this circumstance, autophagy plays a cytoprotective role in apigenin-induced cytotoxicity in cancer cells.

Other immune responses have been reported, studies with human and mouse mammary carcinoma cells developed by Coombs *et al*. (2016) demonstrated that apigenin could target STAT1, causing inhibition of PD-L1 expression induced by IFN-*γ*. Meanwhile, apigenin treatment induced the proliferation of Jurkat T cells PD1-expressing and interleukin-2 synthesis when co-cultured with MDA-MB-468 cells. According to a previous study conducted by Kuo *et al*., (1992) apigenin is capable of reversing mutations in *Salmonella* T98 strains and hamster ovary cells. This is due to a possible induction of a cellular defense system, through metabolizing enzymes such as glutathione-S-transferase (GST) or excision repair enzymes. Babcook & Gupta (2012) suggest that apigenin acts to downregulate insulin-like growth factor 1 (IGF-1R), which is the predominant receptor in mitogenesis, transformation, and protection from apoptosis. Apigenin instead allows IGF-1R to form a protein complex with IGFBP-3, which regulates cell proliferation through competitive inhibition (Burger *et al*., 2005) (Figure 1a).

Artocarpin is capable of generating cytotoxicity in cancer cells

Tsai *et al*. (2017) reported that artocarpin has a cytotoxic effect on cancer cells by inducing the production of reactive oxygen species. Mainly hydrogen peroxide (H_2O_2) is formed during the generation of the superoxide anion radical (O_2^-), which allows the formation of the OH radical. Zhou *et al*. (2006) informed that H_2O_2 induces apoptosis in MCF-7 breast cancer cells mediated by the phosphorylation of MARK, ERK, p28, and JNK proteins. The H_2O_2 promotes the expression

Revista Bio ciencias
ISSN 2007-3380

of the MKP-1 protein. MKP-1 is an early stress response protein that inhibits the activation of apoptosis by p38 and JNK pathways. In addition, they allow the activation of the P53 protein, being the main responsible for triggering apoptosis through the caspase pathway (Figure 1b). In another study, the mechanism of inhibition of HDGF (a nuclear protein with mitogenic activity) was determined by small interfering RNA (Shik *et al*., 2013). These authors propose that the increase in ROS contributes to apoptosis by improving the activation of p53 and PUMA expression.

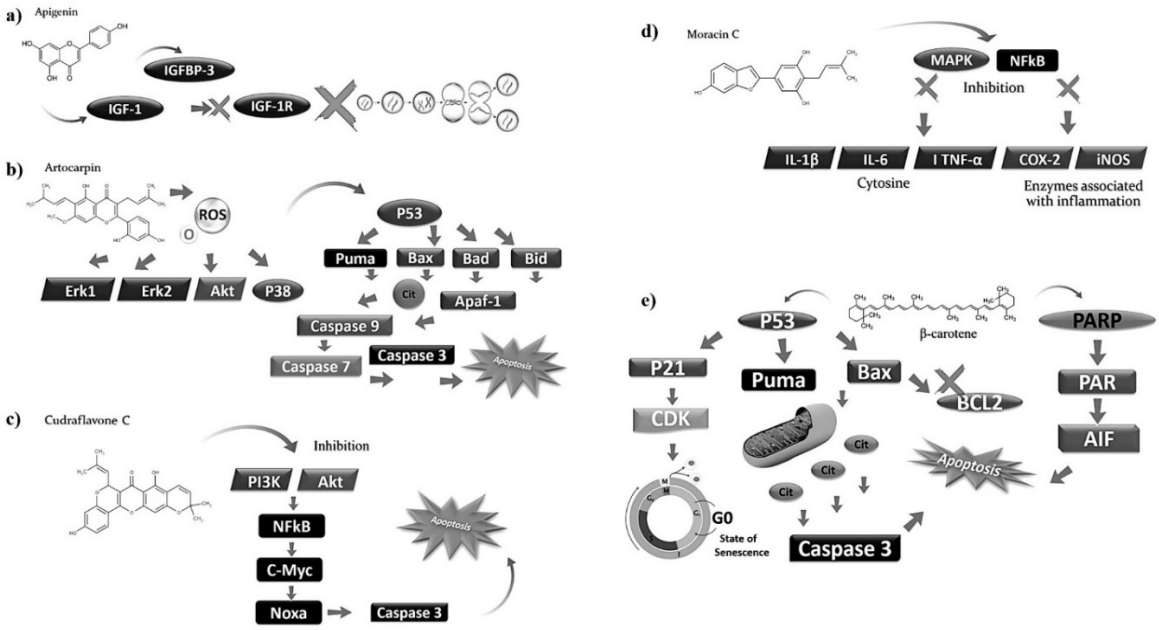

Figure 1. Possible mechanisms of action for the carcinogenesis inhibition of a) apigenin, b) artocarpin, c) cudraflavone C, d) moracin C and e) *β*-carotene.

Cudraflavone C selectively induces apoptosis in cancer cells by inhibiting the PI3K-AKT pathway

Soo *et al*. (2017) found that cudraflavone C could inhibit the PI3K-AKT pathway. This pathway modulates the regulation of cell survival, cell cycle progression, and cell growth. The abnormal activation of the PI3K pathway causes the alteration of the mechanisms that control cell growth and survival. This behavior favors competitive growth, metastatic capacity, and often greater resistance to drug treatments (Carnero *et al*., 2008). Besides, cudraflavone C could cause apoptosis in the PI3K-AKT pathway through the activation of NFκB and induce a signaling cascade that ends in caspase 3 (Figure 1c).

Revista Bio ciencias
ISSN 2007-3380

Moracin C counteracts inflammatory activity in the cancer process through the inhibition of the NF-κB and MAPK pathways

According to Yao *et al*. (2016), this flavonoid can inhibit the NF-κB and MAPK pathways, being the two main mechanisms to produce inflammatory cytokines initiated by lipopolysaccharides (LPS), such as IL-1*β*, IL-6, and TNF-*α* encoding cytokines, as well as inflammation-associated enzymes including COX-2 and iNOS (Figure 1d).

β-carotene induces apoptosis through p53 and PARP

According to Sowmya *et al*. (2017), *β*-carotene mainly acts on two regulatory pathways, such as; a) through the p53 gene, which activates the transcription of the p21 gene. This gene inhibits cyclin-dependent kinases in the G1 phase, leading the cell to a state of senescence in G0. P53 also activates the transcription of the BAX and PUMA genes. This allows the release of cytochromes in the mitochondria, which in turn trigger a series of events, proapoptotic. These events, with the activation of caspase 3, promote apoptosis or programmed cell death. b) poly-ADP-ribose-polymerase can induce apoptosis, through the production of poly-ADP-ribose, which stimulates the release of apoptosis-inducing factor (Figure 1e).

Conclusions

In this study was possible to extract and identify 21 compounds (organic acids, phenolics, carotenoids, and flavonoids) from the methanolic fraction of jackfruit extract. Five compounds such as; *β*-carotene, moracin C, apigenin, artocarpin, and cudraflavone C, may be responsible for the carcinogenesis process inhibition. Based on the compounds identified in the jackfruit extract and reports of their anticancer activity, it was possible to propose postulates about the possible mechanisms of action for the inhibition of carcinogenesis by the HVBCs.

Author contribution

Work conceptualization, JARS; Methodology, JORA, JCBC; Experimental validation, MCS, JARS, JCBC; Analysis of results, JORA, JARS, JCBC; Writing – review & editing, JORA, MCS, JARS, JCBC; Funding acquisition, JARS.

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Conflict of interest

The authors declare no conflict of interest.

References

Babcook, M. A., & Gupta, S. (2012). Apigenin Modulates Insulin-like Growth Factor Axis: Implications for Prevention and Therapy of Prostate Cancer. *Current Drug Targets*. https://doi.org/10.1016/j.immuni.2010.12.017.Two-stage

Burger, A. M., Leyland-Jones, B., Banerjee, K., Spyropoulos, D. D., & Seth, A. K. (2005). Essential roles of IGFBP-3 and IGFBP-rP1 in breast cancer. *European Journal of Cancer*, *41*(11), 1515–1527. https://doi.org/10.1016/j.ejca.2005.04.023

Carnero, A., Blanco-Aparicio, C., Renner, O., Link, W., & Leal, J. (2008). The PTEN/PI3K/AKT Signalling Pathway in Cancer, Therapeutic Implications. *Current Cancer Drug Targets*, *8*(3), 187–198. https://doi.org/10.2174/156800908784293659

Coombs, M. R. P., Harrison, M. E., & Hoskin, D. W. (2016). Apigenin inhibits the inducible expression of programmed death ligand 1 by human and mouse mammary carcinoma cells. *Cancer Letters*, *380*(2), 424–433. https://doi.org/10.1016/j.canlet.2016.06.023

Fang, S. C., Hsu, C. L., & Yen, G. C. (2008). Anti-inflammatory effects of phenolic compounds isolated from the fruits of *Artocarpus heterophyllus*. *Journal of Agricultural and Food Chemistry,* 56(12), 4463-4468. https://doi.org/10.1021/jf800444g

Kuo, M. L., Lee, K. C., & Lin, J. K. (1992). Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in the *Salmonella* and CHO systems. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *270*(2), 87–95. https://doi.org/10.1016/0027-5107(92)90119-M

Ranasinghe, R. A. S. N., Maduwanthi, S. D. T., & Marapana, R. A. U. J. (2019). Nutritional and Health Benefits of Jackfruit (*Artocarpus heterophyllus* Lam.): A Review. *International Journal of Food Science*, *2019*, 4327183. https://doi.org/10.1155/2019/4327183

Ruiz-Montañez, G., Burgos-Hernández, A., Calderón-Santoyo, M., López-Saiz, C. M., Velázquez-Contreras, C. A., Navarro-Ocaña, A., & Ragazzo-Sánchez, J. A. (2015). Screening antimutagenic and antiproliferative properties of extracts isolated from Jackfruit pulp (*Artocarpus heterophyllus* Lam). *Food Chemistry*, *175*, 409–416. https://doi.org/10.1016/j.foodchem.2014.11.122

Shik, H., Hong, E., Lee, S., Baek, J., Lee, C., Yim, J., Um, H., & Hwang, S. (2013). Biochemical and Biophysical Research Communications Depletion of hepatoma-derived growth factor-related protein-3 induces apoptotic sensitization of radioresistant A549 cells via reactive oxygen species-dependent p53 activation. *Biochemical and Biophysical Research Communications*, *439*(3), 333–339. https://doi.org/10.1016/j.bbrc.2013.08.086

Shukla, S., Fu, P., & Gupta, S. (2014). Apigenin induces apoptosis by targeting inhibitor of apoptosis proteins and Ku70-Bax interaction in prostate cancer. *Apoptosis*, 19, 883–894 https://doi.org/10.1007/s10495-014-0971-6

Soo, H. C., Chung, F. F. L., Lim, K. H., Yap, V. A., Bradshaw, T. D., Hii, L. W., Tan, S. H., See, S. J., Tan, Y. F., Leong, C. O., & Mai, C. W. (2017). Cudraflavone C induces tumor-specific apoptosis in colorectal cancer cells through inhibition of the phosphoinositide 3-kinase (PI3K)-AKT pathway. *PLoS ONE*, *12*(1), e0170551. https://doi.org/10.1371/journal.pone.0170551

Sowmya Shree, G., Yogendra Prasad, K., Arpitha, H. S., Deepika, U. R., Nawneet Kumar, K., Mondal, P., & Ganesan, P. (2017). β-carotene at physiologically attainable concentration induces apoptosis and down-regulates cell survival and antioxidant markers in human breast cancer (MCF-7) cells. *Molecular and Cellular Biochemistry*, *436*(1–2). https://doi.org/10.1007/s11010-017-3071-4

Sun, G., Zheng, Z., Lee, M. H., Xu, Y., Kang, S., Dong, Z., Wang, M., Gu, Z., Li, H., & Chen, W. (2017). Chemoprevention of Colorectal Cancer by Artocarpin, a Dietary Phytochemical from *Artocarpus heterophyllus*. *Journal of Agricultural and Food Chemistry*, *65*(17), 3474-3480. https://doi.org/10.1021/acs.jafc.7b00278

Tong, X., Smith, K. A., & Pelling, J. C. (2012). Apigenin, a chemopreventive bioflavonoid, induces AMP-activated protein kinase activation in human keratinocytes. *Molecular Carcinogenesis*, *51*(3), 268–279. https://doi.org/10.1002/mc.20793

Tsai, M.-H., Liu, J.-F., Chiang, Y.-C., Hu, S. C.-S., Hsu, L.-F., Lin, Y.-C., Lin, Z.-C., Lee, H.-C., Chen, M.-C., Huang, C.-L., & Lee, C.-W. (2017). Artocarpin, an isoprenyl flavonoid, induces p53-dependent or independent apoptosis via ROS-mediated MAPKs and Akt activation in non-small cell lung cancer cells. *Oncotarget*, *8*(17), 28342–28358. https://doi.org/10.18632/oncotarget.16058

Witsch, E., Sela, M., & Yarden, Y. (2010). Roles fr Growth Factors in Cancer Progression. *Physiology*, *25*(2), 85–101. https://doi.org/10.1152/physiol.00045.2009

Yan, X., Qi, M., Li, P., Zhan, Y., & Shao, H. (2017). Apigenin in cancer therapy: Anti-cancer effects and mechanisms of action. *Cell and Bioscience*, *7*, 50. https://doi.org/10.1186/s13578-017-0179-x

Yao, X., Wu, D., Dong, N., Ouyang, P., Pu, J., Hu, Q., Wang, J., Lu, W., & Huang, J. (2016). Moracin C, A phenolic compound isolated from *Artocarpus heterophyllus,* suppresses lipopolysaccharide-activated inflammatory responses in murine raw264.7 macrophages. *International Journal of Molecular Sciences*, *17*(8), 1199. https://doi.org/10.3390/ijms17081199

Zhang, Y., Zhang, Y., Xu, F., Li, S., & Tan, L. (2018). Structural characterization of starches from Chinese jackfruit seeds (*Artocarpus heterophyllus* Lam). *Food Hydrocolloids*, *80*, 141–148. https://doi.org/10.1016/j.foodhyd.2018.02.015

Zheng, Z. P., Xu, Y., Qin, C., Zhang, S., Gu, X., Lin, Y., Xie, G., Wang, M., & Chen, J. (2014). Characterization of antiproliferative activity constituents from *Artocarpus heterophyllus*. *Journal of Agricultural and Food Chemistry*, *62*(24), 5519–5527. https://doi.org/10.1021/jf500159z

Zhou, J., Liu, Y., & Wu, G. S. (2006). *The Role of Mitogen-Activated Protein Kinase Phosphatase-1 in Oxidative Damage – Induced Cell Death*.66 (*9*), 4888–4895. https://doi.org/10.1158/0008-5472.CAN-05-4229