PHARMACON
Jurnal Farmasi Indonesia
Pharmaceutical Journal of Indonesia

Pharmacon: Jurnal Farmasi Indonesia
Vol. 21 No. 2 (2024), pp.154-160
DOI: 10.23917/pharmacon.v21i2.5686
p-ISSN: **1411-4283**, e-ISSN: **2685-5062**

Review Selaginella's Potential for Anticancer

Rosa Sayentina Amin[1], Faghfirlie[2], Maryati[3*], Cita Primada Hari Anugrahini[4]

[1-4] *Faculty of Pharmacy, University of Muhammadiyah Surakarta, Indonesia*

**Corresponding author: maryati@ums.ac.id*

ARTICLE HISTORY:
Submitted : 2024-07-01
Accepted : 2024-12-30
Published : 2024-12-31

KEYWORDS:
Amentoflavon; Anticancer; Co-chemotherapy; Doxorubicin; Selaginella

Citation:
Amin, R. S., Faghfirlie, Maryati, Anugrahini, C. P. H. (2024). Review Selaginella's Potential for Anticancer. *Pharmacon: Jurnal Farmasi Indonesia,* 21(2), 154–160. https://doi.org/10.23917/pharmacon.v21i2.5686

ABSTRACT

Selaginella is a popular herbal remedy for its antioxidants, anticancer, anti-inflammatory, and chemopreventive properties. Several studies have reported Selaginella's potential as an anticancer and co-chemotherapy agent. This narrative review aims to investigate Selaginella's potential as an anticancer agent and co-chemotherapy with doxorubicin. Studies were retrieved from PubMed, Science Direct, and Google Scholar databases. In total, 27 articles were selected. The results showed that Selaginella extract had cytotoxic activity against cancer cells T47D, MCF-7, A549, LLC, HeLa, Bel-7402, HT-29, 7721, P-388, and NCI-H460. One of the compounds contained and studied its mechanism is amentoflavone. Amentoflavone induces cell cycle arrest and apoptosis in MCF-7 cells. Amentoflavone also inhibited invasion and migration of A549 and HT29 cancer cells. The combination with doxorubicin indicates that Selaginella and amentoflavone extracts could increase the anticancer effects of doxorubicin in vivo by decreasing the tumor volume in the cancer cell-induced animals. These results demonstrate Selaginella's potential as a chemotherapeutic agent to enhance the anticancer effects of doxorubicin. Nevertheless, further research is necessary to have more insights and evidence regarding its activity.

INTRODUCTION

Cancer, which can affect any organ or body region, is defined by abnormal cell proliferation (Doll, 2018). After heart disease and stroke, cancer is the leading killer in Indonesia. In 2018, 9.6 million people worldwide were cancer patients. In contrast, there were 207,210 patients in Indonesia. As people's lives change, the risk of developing cancer increases. It is predicted that the number of cancer cases worldwide will increase by 300 percent by the year 2030, with the majority of cases occurring in emerging economies such as Indonesia (Kemenkes RI, 2016).

Chemotherapy, radiation, and surgery are currently available cancer treatments. The most common treatment is chemotherapy (Hartini et al., 2020). One of the often-used chemotherapy drugs is doxorubicin, although it has risks, including side effects that can be damaging to the heart and liver as well as a chance of developing resistance (Minotti et al., 2004). Therefore, it is important to reduce these adverse effects and boost doxorubicin's anticancer efficacy. The cytotoxic effect of doxorubicin is predicted to be enhanced in combination with co-chemotherapeutic drugs from natural components.

A variety of pharmacologically active plants are among Indonesia's natural resources. Plants like *Selaginella* have pharmacological properties, including anticancer, antiviral, and anti-inflammatory effects. Indonesia has 200 of the 700-750 *Selaginella* species (Setyawan, 2009). The *Selaginella* plant is used as a medicinal herb in nations like India and China for its antioxidant, anticancer, anti-inflammatory, and chemopreventive characteristics because it doesn't harm healthy cells (G. Wang et al., 2015).

There have been many studies reporting the activity of *Selaginella* as an anticancer agent, but there has not been a comprehensive review on this topic. This narrative review aims to investigate *Selaginella*'s potential as an anticancer agent and co-chemotherapy with doxorubicin.

METHODS

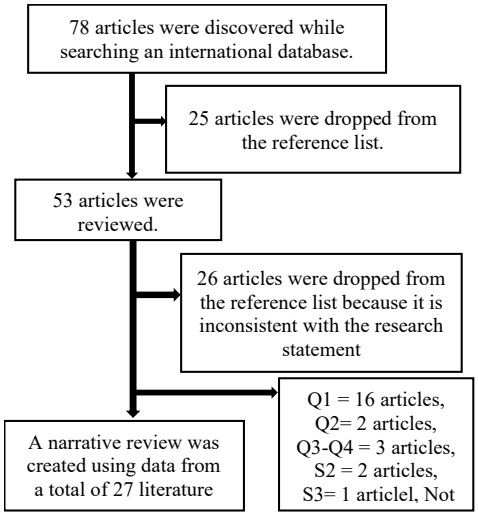


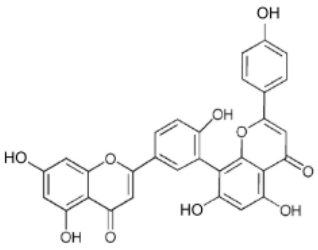
Figure 1. Article Search Scheme for Narrative Review Preparation

This narrative review was prepared using the method of analysis of secondary data (ADS). ADS is a research method that refers to a systematic review of records and uses secondary data as the primary data source. PRISMA requires systematic reviews (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). A literature search was conducted using the keywords "*Selaginella* as anticancer," "*Selaginella* doxorubicin," "Apigenin *Selaginella*," "Robustaflavone *Selaginella* anticancer," "Amentoflavone *Selaginella* anticancer," "Cytotoxic test used for," "Metastasis is the ability of cells," and "Combination of chemotherapy and *Selaginella* chemoprevention compounds." There were 78 articles returned in the search results. Articles were selected and stored in Mendeley. A total of 25 journals were excluded because the titles did not match the topic and were published before 2004. Further review of 53 journals on the anticancer potential of different *Selaginella* species and compounds and on the combination of doxorubicin and *Selaginella*. In all, 26 journals were excluded because they did not fit the research topic. This narrative review was prepared using a total of 27 journals. There are 16 journals in the Quartile 1 (Q1) category, 2 journals in the Q2 category, 3 journals in the Q3-Q4 category, 2 Sinta 2 (S2) accredited journals, and 1 Sinta 3 (S3) accredited journal (**Figure 1**).

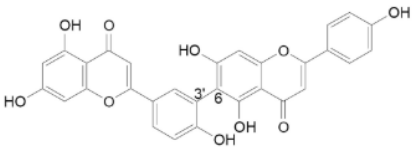
RESULT AND DISCUSSION

Selaginella's Active Components

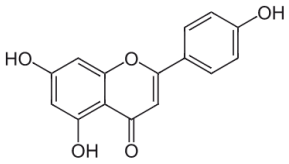
Selaginella sp. contains many bioactive compounds such as flavonoids, alkaloids, and terpenoids (**Figure 2**). Biflavonoids are dimeric forms of flavonoids that are found in *Selaginella.* At least 13 biflavonoid compounds were found in *Selaginella*, namely amentoflavones, 2', 8'-biapigenin, delicaflavones, ginkgetin, heveaflavone, hinokiflavone, isocryptomerin, kayaflavone, ochnaflavone, podocarpusflavone A, robustaflavone, and sumaflavone (Setyawan, 2011). *Selaginella doederleinii* Hieron found amentoflavones, robustaflavones, and 2′′, 3′′-dihydrogen-3′, 3′′′-biapigenin (Sui et al., 2016)



Amentoflavone



Robustaflavone



Apigenin

Figure 2. Selaginella contains anticancer and chemopreventive properties

Selaginella's Anticancer Activity

Cancer is a cell growth disorder that results in the invasion and destruction of normal tissue around the abnormal cells. Cancer arises from normal cells that change their nature permanently (Doll, 2018). Cancer cells have the

following characteristics: (1) Capable of unlimited replication resulting in macroscopic tumors (2) Induce angiogenesis, namely the formation of new blood vessels (3) Able to avoid cell death (apoptosis) (4) Able to maintain proliferation signals (5) Avoid anti-growth signals (6) Activate invasion and metastasis (Hanahan & Weinberg, 2011). Anticancer compounds are substances that can either inhibit or kill cancer cells. Cytotoxic tests are commonly used to assess a compound's ability to kill cancer cells. Anticancer compounds are substances that can either inhibit or kill cancer cells. Cytotoxic tests are commonly used to assess a compound's ability to kill cancer cells (Wisnu,. et. al, 2017). NCI (National Cancer Institute) determines the cytotoxic criteria of a compound based on the IC_{50} value. IC_{50} is the sample concentration required to inhibit cell growth by 50%. According to the NCI, a compound is declared to have potential as an anticancer, if IC_{50} value is 20µg/mL. Until now, many studies have been carried out on the anticancer potential of various *Selaginella* species **Table 1** and **Error! Reference source not found.**.

Cancer Invasion and Metastasis

Metastatic disease is the spread of cancer cells throughout the body (Febriani & Furqon, 2018). In vitro, *S. tamariscina* ethanolic extract inhibited the invasion and metastasis of LLC cells. The in vivo tests also showed that the extract could reduce the expression of matrix metalloproteinases (MMP)-2, -9, and urokinase in LLC cancer cells in C57BL/6 mice (u-PA) (Yang et al., 2007). In addition, *S. tamariscina* amentoflavonoids have been shown to inhibit invasion and migration of A549 and HT-29 cancer cells in vitro and in vivo by inhibiting TGF-β -induced EMT (Kim et al., 2020). EMT is involved in cancer metastasis. Its participation in other events may be highly relevant to tumor progression (Thiery et al., 2009). Apigenin, a novel flavonoid, was discovered by phytochemical screening. Apigenin showed significant activity against A549 cancer cells in in vitro cytotoxicity tests (Zou et al., 2017). In vitro, *S. delicatula* robustaflavone demonstrated cytotoxic activity against P-388 and HT-29 (Chen et al., 2005) (**Table 3**).

Table 1. *Selaginella* Extract and Essential Oil Protect Against a Variety of Cells

***Selaginella*'s species**	**Cell**	**IC_{50} (µg/mL)**	**Mechanisms of Action**	**Reference**
***S. tamariscina* (Ethanol extract)**	LLC	-	Inhibits cell invasion and metastasis	(Yang et al., 2007)
***S. tamariscina* (Ethyl acetate extract)**	HT-29	3,24	Induces cell apoptosis	(J. Li et al., 2014)
***S. labordei* (Ethyl acetate extract)**	Bel-7402	1,07		(J. Li et al., 2014)
***S. uncinata* (Ethyl acetate extract)**	HeLa	15,14		(J. Li et al., 2014)
***S.plana* Hieron (Ethanol extract)**	T47D	4,00		(Handayani et al., 2012)
Essential oil *S. doederleinii*	7721	-	Inhibits cell proliferation	(G. Wang et al., 2015)

Table 2. *Selaginella* Extract's Anticancer Activity in Vivo

***Selaginella*'s Species**	**Cancer Type**	**Animals Used In Research**	**Mechanisms of Action**	**Reference**
***S. doederleinii* (Ethyl acetate extract)**	Lung	C57BL/6 Rat (male)	Inhibit tumor growth	(Yao et al., 2017)
***S. doederleinii* (Ethyl acetate extract)**	Liver	Kunming Rat (male)	Inhibits proliferation and induces cell apoptosis	(Wang et al., 2015)
***S. tamariscina* (Ethanol**	Lung	C57BL/6 mice	Inhibits cancer	(Yang et al., 2007)

Table 3. Anticancer Mechanism of Amentoflavones, Robustaflavones, and Apigenin

Chemical Compound	Physiological/cellular effects as anticancer	Molecular Mechanism	Reference
Amentoflavon (AF)	It inhibits A549 and HT-29 cancer cell invasion and migration in vitro and in vivo.	TGF- activation is inhibited, resulting in the restoration of E-cadherin expression.	(Kim *et al.*, 2020)
	It inhibits the proliferation of A549 and NCI-H460 cells.	AKR1B10 mRNA and protein expression is increased.	(Jung *et al.*, 2017)
	Cell-cycle arrest and apoptosis are induced in MCF-7 breast cancer cells.	Caspase activation and cytochrome c release from mitochondria.	(Pei *et al.*, 2012)
Robustaflavon	In vitro, it has cytotoxic activity against P-388 and HT-29 cells.	-	(Chen *et al.*, 2005)
Apigenin	In vitro, it has cytotoxic activity against A549 cancer cells.	-	(Zou *et al.*, 2017)

Selaginella Sonnei Inhibits Proliferation and Induces Apoptosis

S. Tamariscina, S. Labordei, and *S. Uncinata* have been shown to induce apoptosis in HeLa, Bel-7402, and HT-29 cells (J. Li et al., 2014). The ethanolic extract of *S. plana Hieron* induces apoptosis in T47D cells with an IC_{50} value of 4 g/mL (Handayani et al., 2012). *S. doederleinii* ethyl acetate extract had no toxic effect on normal cells and inhibited the growth of liver cell tumors in Kunming (male) rats in vivo. This extract has also been shown to reduce the ratio of bcl-2 to bax mRNA levels, activate caspase-3, suppress survivin, and decrease COX-2, 5-LOX, FLAP, and 12-LOXmRNA gene expression (J. Z. Wang et al., 2015). Oral administration of S. doederleinii ethyl acetate extract at doses of 50 mg/kg/day and 150 mg/kg/day significantly ($p < 0.01$) inhibited the growth of LLC cells (lung cancer) and inhibited tumor growth by 40.11 percent and 53.5 percent, respectively, for both doses tested (Yao et al., 2017). *S. doederleinii Hieron*'s essential oil content has also been tested for cytotoxic activity against cancer cells. The essential oil has been shown to inhibit cell proliferation and induce apoptosis in 7721 and A5491 cancer cells (J. Z. Wang et al., 2015).

Selaginella as a Potential Co-Chemotherapy Against Doxorubicin

Improving the anticancer effectiveness of doxorubicin is the goal of using chemopreventive agents. Doxorubicin is a chemotherapy agent commonly used to treat breast cancer. However, doxorubicin was found to be ineffective, and some T47D breast cancer cells were resistant to it (Smith et al., 2006). According to research, doxorubicin causes hepatotoxicity and cardiotoxicity (Minotti et al., 2004). Doxorubicin can be combined with natural chemopreventive compounds to address this issue. This method is known as co-chemotherapy. Co-chemotherapy can increase the efficacy of a chemotherapy drug, allowing lower doses to be used. Lowering the dose is expected to reduce toxicity in normal tissues.

Doxorubicin resistance increased Akt phosphorylation activity in T47D cancer cells. Phosphorylated Akt activates Bcl-XL (antiapoptotic protein) and inactivates caspase-9 (initiator of apoptosis) via the caspase pathway (X. Li et al., 2005). Doxorubicin can be combined with co-chemotherapeutic agents to overcome this problem. The combination of *S. tamariscina* extract and doxorubicin demonstrated a synergistic antitumor effect in mice (BALB/c). Doxorubicin's anticancer effect can be enhanced by *S. tamariscina* extract (Lee et al., 2017).

In vivo experiments on BALB/c mice induced by A549 cancer cells revealed that combining S. tamariscina extract (STE) and doxorubicin resulted in a significant reduction in tumor volume. A549 and NCI-H460 cells are inhibited in their proliferation by mentoflavone compounds. Amentoflavones increase antiproliferative effects of doxorubicin against A549 and NCI-H460 cells (human lung cancer cells) by inhibiting AKRB10 activity and decreasing AKRB10 mRNA expression (Jung et al., 2017). AKR1B10 is an aldo-keto reductase enzyme involved in drug resistance mechanisms. The AKR1B10 enzyme is abundant in breast

cancer (Ma et al., 2012). Amentoflavones induce cell-cycle arrest and apoptosis in MCF-7 breast cancer cells through a series of apoptosis-associated cellular changes, including DNA and nuclear fragmentation, as well as de-regulation of intracellular reactive oxygen species (ROS) and calcium. These findings suggest that amentoflavones could be used as co-chemotherapeutic agents in the treatment of breast cancer (Pei et al., 2012). Natural compounds used as cancer co-chemotherapy can reduce cell resistance to anticancer agents while also increasing a compound's anticancer activity. Furthermore, co-chemotherapy agents can reduce the side effects of anticancer agents (Vinod et al., 2013).

CONCLUSIONS

There are five *Selaginella* species that have anticancer potential, namely *S. tamariscina S. Labordei S. uncinata* Hieron plana and *S. doederleinii. S. Labordei* has the greatest anticancer and co-chemotherapy potential. The main chemical constituents responsible for *Selaginella*'s anticancer activity are amentoflavones. *Selaginella* has the potential to be developed as a co-chemotherapy agent to boost doxorubicin's anticancer activity while reducing the risk of side effects.

ACKNOWLEDGMENT

The authors are grateful to the Ministry of Education and Culture's Directorate General of Learning and Student Affairs for funding this research.

AUTHORS' CONTRIBUTIONS

The research concept and study design were performed by Rosa Sayentina Amin, Maryati; all authors provided administrative technical/ logistical support and performed data collection; validation and supervision critical analysis of the article for important intellectual content was performed by Maryati, all authors contributed to the drafting of the article and approved the final version.

CONFLICT OF INTERESTS

The authors have no conflict of interests related to this publication.

ETHICAL CONSIDERATION

Ethical issues (including plagiarism, data fabrication, double publication, etc) have been completely observed by the authors**.**

BIBLIOGRAPHY

Chen, J. J., Duh, C. Y., & Chen, J. F. (2005). New cytotoxic biflavonoids from Selaginella delicatula. *Planta Medica*, *71*(7), 659–665. https://doi.org/10.1055/s-2005-871273

Doll, R. (2018). The Cancer Process. *The Prevention of Cancer*, 110–117. https://doi.org/10.4324/9781315134178-7

Febriani, A., & Furqon, A. (2018). Metastasis Kanker Paru. *Jurnal Respirasi*, *4*(3), 94–101.

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, *144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Handayani, S., Risdian, C., Meiyanto, E., Udin, Z., Andriyani, R., & Angelina, M. (2012). Selaginella Active Fractions Induce Apoptosis On T47d Breast Cancer Cell. *SciFed Dental & Oral Research Journal*, *1*(4), 48–53. https://doi.org/10.23959/sfdorj-1000023

Hartini, S., Winarsih, B. D., Galih, E., Nugroho, Z., Studi, P., & Ners, P. (2020). *Peningkatan Pengetahuan Perawat Untuk Perawatan Anak Penderita Kanker*. *3*(2), 141–149. https://doi.org/10.31596/jpk.v3i2.87

Jung, Y. J., Lee, E. H., Lee, C. G., Rhee, K. J., Jung, W. S., Choi, Y., Pan, C. H., & Kang, K. (2017). AKR1B10-inhibitory Selaginella tamariscina extract and amentoflavone decrease the growth of A549 human lung cancer cells in vitro and in vivo. *Journal of Ethnopharmacology*, *202*, 78–84. https://doi.org/10.1016/j.jep.2017.03.010

Kim, G. L., Jang, E. H., Lee, D. E., Bang, C., Kang, H., Kim, S. H., Yoon, S. Y., Lee, D. H., Na, J. H., Lee, S., & Kim, J. H. (2020). Amentoflavone, active compound of Selaginella tamariscina, inhibits in vitro and in vivo TGF-β-induced metastasis of human cancer cells. *Archives of Biochemistry and Biophysics*, *687*(January), 108384. https://doi.org/10.1016/j.abb.2020.108384

Lee, C. G., Lee, E. H., Pan, C. H., Kang, K., & Rhee, K. J. (2017). Data on the anti-tumor effects of Selaginella tamariscina extract and amentoflavone combined with doxorubicin in mice. *Data in Brief*, *13*(May), 162–165. https://doi.org/10.1016/j.dib.2017.05.023

Li, J., Lei, X., & Chen, K. L. (2014). Comparison of cytotoxic activities of extracts from Selaginella species. *Pharmacognosy Magazine*, *10*(40), 529–535. https://doi.org/10.4103/0973-1296.141794

Li, X., Lu, Y., Liang, K., Liu, B., & Fan, Z. (2005). Differential responses to doxorubicin-induced phosphorylation and activation of Akt in human breast cancer cells. *Breast Cancer Research*, *7*(5). https://doi.org/10.1186/bcr1259

Ma, J., Luo, D. X., Huang, C., Shen, Y., Bu, Y., Markwell, S., Gao, J., Liu, J., Zu, X., Cao, Z., Gao, Z., Lu, F., Liao, D. F., & Cao, D. (2012). AKR1B10 overexpression in breast cancer: Association with tumor size, lymph node metastasis and patient survival and its potential as a novel serum marker. *International Journal of Cancer*, *131*(6), 1–10. https://doi.org/10.1002/ijc.27618

Minotti, G., Menna, P., Salvatorelli, E., Cairo, G., & Gianni, L. (2004). Anthracyclines: Molecular advances and pharmacologie developments in antitumor activity and cardiotoxicity. *Pharmacological Reviews*, *56*(2), 185–229. https://doi.org/10.1124/pr.56.2.6

Pei, J. S., Liu, C. C., Hsu, Y. N., Lin, L. L., Wang, S. C., Chung, J. G., Bau, D. T., & Lin, S. S. (2012). Amentoflavone induces cell-cycle arrest and apoptosis in MCF-7 human breast cancer cells via mitochondria-dependent pathway. *In Vivo*, *26*(6), 963–970.

Setyawan, A. D. W. I. (2009). Traditionaly utilization of Selaginella; field survey and literature review. *Nusantara Bioscience*, *1*(3), 146–158. https://doi.org/10.13057/nusbiosci/n010307

Setyawan, A. D. W. I. (2011). Natural products from Genus Selaginella (Selaginellaceae). *Nusantara Bioscience*, *3*(1), 44–58. https://doi.org/10.13057/nusbiosci/n030107

Smith, L., Watson, M. B., O'Kane, S. L., Drew, P. J., Lind, M. J., & Cawkwell, L. (2006). The analysis of doxorubicin resistance in human breast cancer cells using antibody microarrays. *Molecular Cancer Therapeutics*, *5*(8), 2115–2120. https://doi.org/10.1158/1535-7163.MCT-06-0190

Sui, Y., Li, S., Shi, P., Wu, Y., Li, Y., Chen, W., Huang, L., Yao, H., & Lin, X. (2016). Ethyl acetate extract from Selaginella doederleinii Hieron inhibits the growth of human lung cancer cells A549 via caspase-dependent apoptosis pathway. *Journal of Ethnopharmacology*, *190*, 261–271. https://doi.org/10.1016/j.jep.2016.06.029

Thiery, J. P., Acloque, H., Huang, R. Y. J., & Nieto, M. A. (2009). Epithelial-Mesenchymal Transitions in Development and Disease. *Cell*, *139*(5), 871–890. https://doi.org/10.1016/j.cell.2009.11.007

Vinod, B. S., Maliekal, T. T., & Anto, R. J. (2013). Phytochemicals as chemosensitizers: from molecular mechanism to clinical significance. *Antioxidants and Redox Signaling*, *18*(11), 1307–1348. https://doi.org/10.1089/ars.2012.4573

Wang, G., Yao, S., Cheng, L., Luo, Y., & Song, H. (2015). Antioxidant and anticancer effection of the volatile oil from various habitats of Selaginella doederleinii Hieron. *Technology and Health Care*, *23*(S1), S21–S27. https://doi.org/10.3233/thc-150924

Wang, J. Z., Li, J., Zhao, P., Ma, W. T., Feng, X. H., & Chen, K. L. (2015). Antitumor activities of ethyl acetate extracts from selaginella doederleinii hieron in vitro and in vivo and its possible mechanism. *Evidence-Based Complementary and Alternative Medicine*, *2015*. https://doi.org/10.1155/2015/865714

Yang, S. F., Chu, S. C., Liu, S. J., Chen, Y. C., Chang, Y. Z., & Hsieh, Y. S. (2007). Antimetastatic activities

of Selaginella tamariscina (Beauv.) on lung cancer cells in vitro and in vivo. *Journal of Ethnopharmacology*, *110*(3), 483–489. https://doi.org/10.1016/j.jep.2006.10.010

Yao, H., Chen, B., Zhang, Y., Ou, H., Li, Y., Li, S., Shi, P., & Lin, X. (2017). Analysis of the total biflavonoids extract from Selaginella doederleinii by HPLC-QTOF-MS and its in vitro and in vivo anticancer effects. *Molecules*, *22*(2). https://doi.org/10.3390/molecules22020325

Zou, Z. X., Tan, G. S., Zhang, G. G., Yu, X., Xu, P. S., & Xu, K. P. (2017). New cytotoxic apigenin derivatives from Selaginella doederleinii. *Chinese Chemical Letters*, *28*(5), 931–934. https://doi.org/10.1016/j.cclet.2017.01.011