

Mechanism of Alkaloids, Flavonoids, and Terpenoids from Bajakah Wood Extract (*Uncaria nervosa* Elmer) in Inducing Apoptosis in Cancer Cells

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ABSTRACT

Cancer is a disease characterized by cells that experience abnormal and uncontrolled mechanisms in regulating cell proliferation, differentiation, and apoptosis. According to the Indonesian Basic Health Research in 2018, the prevalence of cancer in Indonesia is still high, namely 1.79 per 1000. One of the treatments for cancer is chemotherapy, but it has side effects that can reduce the patient's quality of life. Therefore, researcher conducted further research on alternative medicine using extract of Bajakah (*Uncaria nervosa* Elmer). This study used the systematic literature review method with 5 journals obtained from the ScienceDirect, PubMed, and Google Scholar databases. The results showed that the the types of alkaloids in the wood extract of Bajakah were rhynchophylline and isorhynchophylline, the most abundant types of fla-

vonoids were flavanols and flavonols, and the most abundant types of terpenoids were ursan. The mechanism of action of alkaloids and flavonoids in general is to induce apoptosis through Tumor Necrosis Factor Receptors (TNFR). Flavonoids, isorhynchophylline, and ursan induce Bax and Bak. Isorhynchophylline and ursan suppress B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL) expression. Also rhynchophylline and isorhynchophylline increase p53 signaling.

Keywords: Alkaloids, Flavonoids, Terpenoids, *Uncaria nervosa* Elmer, Apoptosis

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INTRODUCTION

Cancer is a disease characterized by body cells that experience abnormal and uncontrolled mechanisms in regulating cell survival, proliferation and differentiation (Dark GG, 2013). Cancer is a health problem in many countries in the world and is a disease of serious concern. This is because the prevalence of cancer in Indonesia has increased from 2013 data of 1.4 per 1000 population to 1.79 per 1000 population in 2018 (Riskesdas, 2013; Riskesdas, 2018). Meanwhile, according to WHO, 2020 cancer ranks as the second most common cause of death from non-communicable diseases after cardiovascular disease, which is as many as 9 million or 22%.

Cancer occurs due to uncontrolled cell growth and continuously multiplies and damages the shape and function of the organs where it grows (Sjamsuhidajat R and Jong W, 2017). Cancer is characterized by the accumulation of a number of genetic changes and causes changes in cell function which is called the process of oncogenesis (Hassanpour SH and Dehghani M, 2017). Genetic changes can be in the form of mutations or changes in the DNA code sequence, deletions of DNA sections, DNA amplification, or in the form of epigenetic changes (Lyman GH, *et al.*, 2015). Cancer cells start with DNA damage that causes failure of DNA repair and results in genomic mutations in somatic cells. This causes the activation of oncogene growth triggers, inactivation of tumor suppressor genes, and changes in apoptosis regulatory genes, so that cells experience uncontrolled proliferation and decreased apoptotic activity. This results in cancer which will develop and experience invasion and metastasis (Sjamsuhidajat R and Jong W, 2017). Apoptosis is a programmed cell death mechanism by which cells with DNA damage are eliminated. The three main features of biochemical changes in apoptosis include caspase activation, breakdown of DNA and proteins, and changes in the membrane so that cells that experience DNA damage can be recognized by phagocytic cells (Sari LM, 2018). However, cancer cells have the ability to avoid the mechanism of apoptosis, thus allowing exces-

sive proliferation and can live in hypoxic conditions (Matsuura K, *et al*, 2016).

Cancer treatment is divided into two, namely curative therapy and palliative therapy. Curative therapy is therapy to heal and extend the patient's life. While palliative therapy is therapy to improve the patient's quality of life by eliminating disturbing symptoms and signs, such as pain, insomnia, difficulty defecating, depression, and others. In general, cancer treatment is by using surgery, radiotherapy, or chemotherapy (Sjamsuhidajat R and Jong W, 2017; Pratama FE and Nuwarda RF, 2018). Chemotherapy is an act or therapy of administering chemical compounds to reduce, eliminate, or inhibit the growth of cancer cells. However, the use of chemotherapy in cancer patients is not specific, because chemotherapy drugs not only inhibit the proliferation of cancer cells, but can also affect normal cells. In addition, chemotherapy has drawbacks which will reduce the patient's quality of life such as expensive costs and can cause side effects such as nausea, vomiting, fever, diarrhea, kidney toxicity, alopecia, movement disorders, cognitive deficits, leukopenia, thrombocytopenia, anemia, and mucosal ulceration (Fernando J and Jones R, 2015; Hayati H and Wanda D, 2016).

Based on the description above, the researcher wants to explore more about alternative treatments that can be done to reduce the effects resulting from chemotherapy treatment, namely by using extracts of bajakah wood (*Uncaria nervosa* Elmer) which contains secondary metabolites such as alkaloids, flavonoids, and terpenoids. This is similar to Pratama FE and Nuwarda RF, 2018 and Maulina S, *et al.*, 2019 in their research which stated that bajakah wood has secondary metabolite compounds that have anticancer effects.

MATERIALS AND METHODS

This type of research is a systematic literature review, which is a series of literature studies related to the method of collecting library data where the research object is taken from library sources

(Melfianora M and Si M, 2019). Research using a systematic literature review is a type of research that aims to examine or critically review knowledge, ideas, or findings contained in a literature, and then elaborate on theories and connect them into a framework of thought to become an idea and innovation for a particular topic (Farisi MI, 2012).

The method for collecting data in a systematic literature review is the documentation method. The documentation method is a method of collecting data by searching and digging up data from literary sources related to what is meant in the formulation of the writing problem. Theories, data, ideas, and things that have been obtained from various sources in the literature and concluded, then arranged and connected so that they are mutually sustainable and become a document that is used to answer problems that have been formulated by researchers (Siyoto S and Sodik MA, 2015).

Secondary data collection uses ScienceDirect, PubMed, and Google Scholar database sources with inclusion criteria for a period of 10 years, journals

in Indonesian or English, and the type of journal research article or literature review. The stages of systematic literature review research are described in the following Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) method (Figure 1).

RESULTS AND DISCUSSION

Bajakah is one of about 34 species of plants from the genus *Uncaria* and is spread across tropical countries such as Southeast Asia, Africa and South-east America. In Indonesia, bajakah is found in tropical rain forests in the Kalimantan region (Erwin, 2020). Bajakah is widely used by the public because it has many properties contained in its metabolites such as wound medicine, ulcers, fever, asthma, rheumatism, hyperpyrexia, hypertension, headaches, gastrointestinal disorders, anticancer, anticonvulsants, antidepressants, antithrombotics, and bacterial or fungal infections (Ravipati AS, et al., 2014; Zhang Q, et al., 2015; Yang W, et al., 2020) (Figure 2).

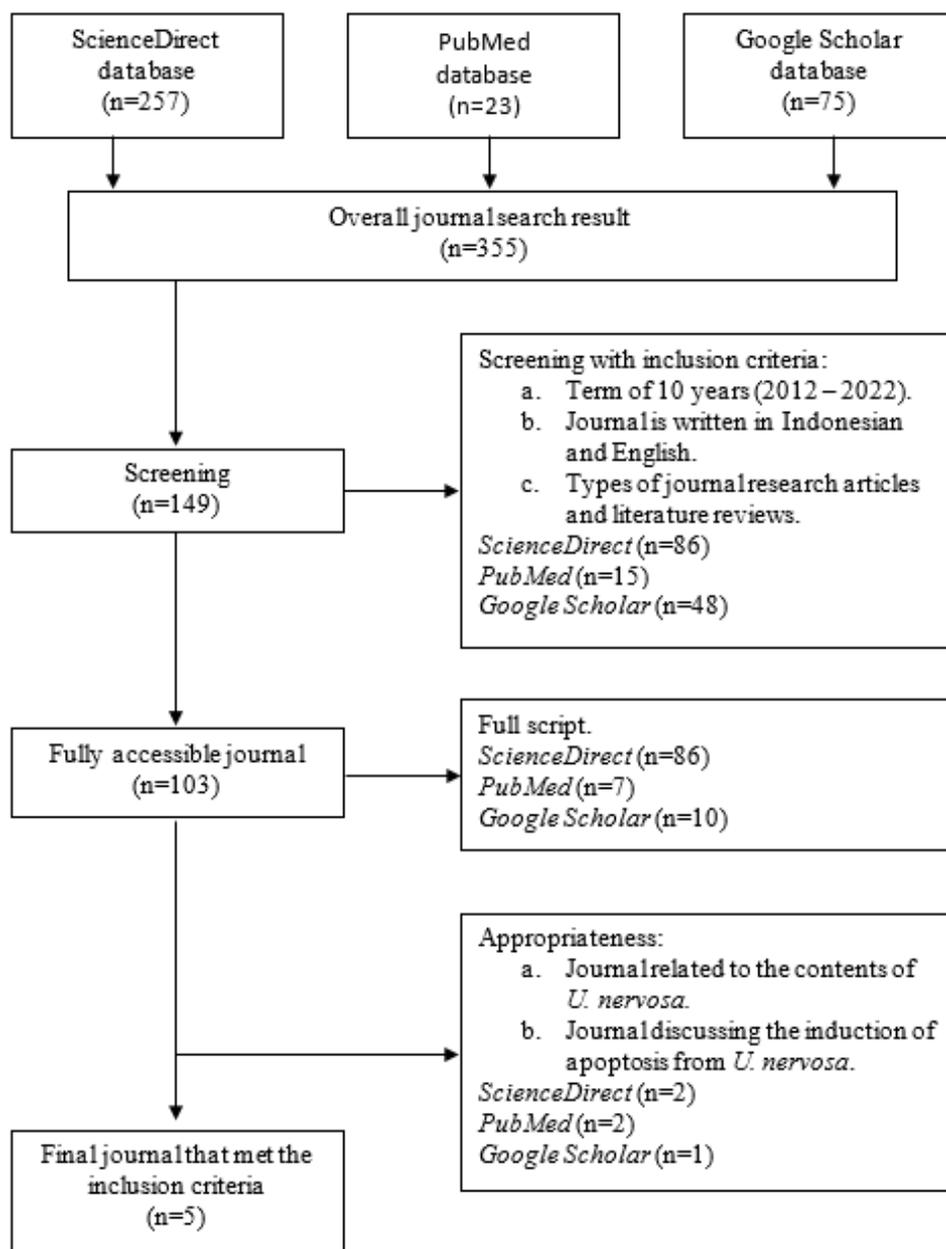


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram



Figure 2: Different parts of Bajakah

Metabolites are substances or compounds of metabolic products produced by an organism (Saroya AS, 2011). The results of the phytochemical tests conducted by Maulina S, *et al.*, 2019 showed that the extract from the bajakah wood contained more secondary metabolites than the bajakah root. The study also stated that the extract of bajakah wood contains alkaloids, flavonoids, and terpenoids. The test results were reinforced by the research of Zhang Q, *et al.*, 2015 and Qin N, *et al.*, 2020 which stated that the secondary metabolites in bajakah contain the highest concentrations of alkaloids, flavonoids, and terpenoids.

Zhang Q, *et al.*, 2015 and Qin N, *et al.*, 2020 stated that alkaloids are the secondary metabolites that are most abundantly contained in bajakah, which is 0.22%. While the types of alkaloids most commonly found in pirates are rhynchophylline and isorhynchophylline (Qin N, *et al.*, 2020; Liang JH, *et al.*, 2020). Qin N, *et al.*, 2020 added that the total content of rhynchophylline and isorhynchophylline is more than 40% of all types of alkaloids present in the pirated plant, with the respective amounts of 28%-50% for rhynchophylline and 15% for isorhynchophylline. In bajakah, the most types of flavonoids are flavanols and flavonols (Zhang Q, *et al.*, 2015; Liang JH, *et al.*, 2020). Meanwhile, the most common types of terpenoids found are triterpenes (Zhang Q, *et al.*, 2015; Maulina S, *et al.*, 2019; Liang JH, *et al.*, 2020). More specifically, the results of research by Ravipati AS, *et al.*, 2014 and Liang JH, *et al.*, 2020 stated that the most common types of triterpenes found were ursan, oleanan, and quinovic. However, in the journal Zhang Q, *et al.*, 2015 stated that ursan type triterpenes were the most common type found in bajakah.

Research by Zhang Q, *et al.*, 2015 stated that alkaloids have effectiveness in inducing apoptosis through Tumor Necrosis Factor Receptors (TNFR). Qin N, *et al.*, 2020 added that rhynchophylline alkaloids can induce apoptosis by increasing the p53 signal which can affect Bcl-2 and TNFR proteins. In the results of research tests by Lee H, *et al.*, 2017 stated that isorhynchophylline alkaloids can inhibit cancer cells by inducing apoptosis through the intrinsic pathway by activating caspase 3, caspase 8, caspase 9, and Poly(ADP-ribose) Polymerase (PARP) cleavage. In addition, isorhynchophylline also suppresses the expression of anti-apoptotic genes such as Bcl-2 and Bcl-xL, as well as increases the expression of pro-apoptotic proteins such as Bax and increases p53 signaling.

Zhang Q, *et al.*, 2015 and Liang JH, *et al.*, 2020 explained that flavonoids in general have biological activity in inhibiting cancer cells by inducing apoptosis. As explained by Veeramuthu D, *et al.*, 2017 that flavonoids will work

by binding to TNFR which is in the mitochondrial membrane and will activate caspase 8. In addition, flavonoids will activate Bax and Bak proteins which are pro-apoptotic regulators. On triterpene bioactivity, Zhang Q, *et al.*, 2015 explained that ursan type triterpenes can induce cancer cells. Qin N, *et al.*, 2020 added, ursan triterpenes have a mechanism to prevent proliferation in cancer cells and induce apoptosis by increasing Bax expression, decreasing Bcl-2, and caspase 3 activation.

Based on the explanation above regarding the bioactivity of each secondary metabolite in inducing apoptosis, it can be concluded that each type of secondary metabolite from bajakah wood extract has a different mechanism of action. Rhynchophylline and isorhynchophylline have activity in increasing p53 signaling (Lee H, *et al.*, 2017; Qin N, *et al.*, 2020). There are two pathways in inducing apoptosis through increasing the p53 signal, namely through the intrinsic pathway by suppressing the anti-apoptotic regulator Bcl-2 and the extrinsic pathway *via* TNFR (Aubrey BJ, *et al.*, 2018). In addition, the TNFR pathway is also induced by alkaloids and flavonoids in general (Zhang Q, *et al.*, 2015). The alkaloids and flavonoids that affect the TNFR will make the Cluster of Differentiation 95 (CD95), Tumor-necrosis factor Related Apoptosis-Inducing Ligand (TRAIL) R1 and R2 receptors bind to intracellular Fas-Associated Death Domain (FADD). Meanwhile, TNF-R1 will bind to TNFR1-Associated Death Domain protein (TRADD). The complex formed is called Death-Inducing Signaling Complex (DISC). This complex will activate caspase 8 which will then cleave Bid. Bid will release cytochrome C which will then bind to Apoptotic peptidase activating factor 1 (Apaf-1) and activate caspase 9, which in turn will activate caspase 3. The activated caspase 3 protein will then carry out apoptosis in cancer cells (Veeramuthu D, *et al.*, 2017; Sari LM, 2018).

In the intrinsic pathway, the metabolites isorhynchophylline, flavonoids, and ursan have a mechanism of action in inducing apoptosis by increasing the expression of Bax and Bak, followed by the mechanism of isorhynchophylline and ursan in suppressing the expression of Bcl-2 and Bcl-xL which can inhibit cytochrome C release (Zhang Q, *et al.*, 2015; Lee H, *et al.*, 2017; Qin N, *et al.*, 2020). By suppressing Bcl-2 and Bcl-xL, Bax and Bak will release cytochrome C which will then bind to Apaf-1 and activate caspase 9. Caspase 9 will activate caspase 3 which will then undergo apoptosis in cancer cells (Sari LM, 2018).

Based on the description of the discussion regarding the mechanism of alkaloids, flavonoids, and terpenoids in the extract of bajakah wood above, it can be described in the scheme of the mechanism in inducing apoptosis below (Figure 3).

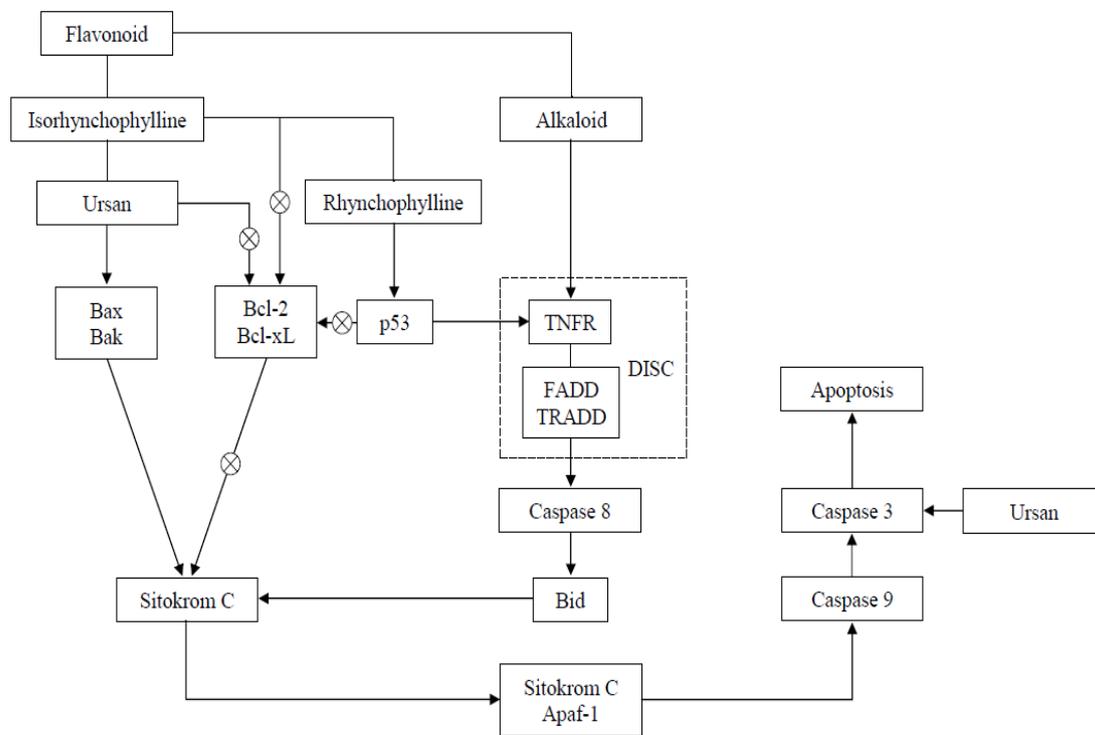


Figure 3: Bajakah secondary metabolite mechanism in inducing apoptosis (Various sources, processed)

In the journals that have been studied by the researchers above, they only describe the mechanism of the secondary metabolites of bajakah wood extract in inducing apoptosis in cancer cells in general, not specifically explained in certain cancer cells. So that further research is needed regarding the mechanism of secondary metabolites from bajakah wood extract in certain cancer cells along with their therapeutic doses.

CONCLUSION

Based on the results of the research review of the literature, it can be concluded that the most common secondary metabolites found in extracts of Bajakah wood are alkaloids of the types rhynchophylline and isorhynchophylline, flavanoids of the flavanol and flavonol types, and terpenoids of the ursan type. All of these secondary metabolites can induce apoptosis in cancer cells through intrinsic and extrinsic pathways.

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