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Potential of *Tithonia diversifolia* Hemsley A. Gray (Kembang Bulan) Leaf Extract as Anti-Cancer Agents

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Abstract

The main objective of this review is to explain the great potential of herbal plants as anticancer agents. Cancer is a disease caused by abnormal cell growth in the body. The high number of cancer incidents still become a global concern because of the high mortality rate. The treatments of cancer such as chemotherapy can cause serious side effects by killing the normal cells. This is the reason why it is necessary to develop an alternative treatment of cancer. I discussed a plant that is believed to has health benefit. Many studies have showed the positive effect of *Tithonia diversifolia* plant for health. After 2000, the researchers discovered a new potential through its cytotoxicity to neoplastic cells. This plant needs to be developed sustainably. However, in the future this plant might become an effective alternative to treat cancer with lower side effects.

Keywords*:* *Tithonia diversifolia* (Hemsley) A. Gray; Kembang bulan; Anticancer.

INTRODUCTION

Cancer is a disease with a multifactorial etiology which is initiated by an abnormal and uncontrolled expression of transcription factors. This can be affected by a gene mutation (Babazadeh et al., 2018). Cancer is one of the main causes of death in the world and still become a global concern. Cancer has the second highest risk of death with a mortality ratio of 1:6 or around 9.6 million deaths in 2018. The most common types of cancer in men are lung, prostate, colorectal, stomach, and liver and the most common types of cancer in women are breast, colorectal, lung, cervical and thyroid cancers (WHO, 2021). Cancer can develop in any part of the body and can spread (metastasize) to other parts of the body. Although some types of cancer can be similar in some ways, how they develop, grow, spread, and respond to drugs can be very different (American Cancer Society, 2021).

The research and development data in 2019 showed the incidence of cancer in Indonesia reached 136.2/100,000 population and reach 8th place in Southeast Asia. The highest incidence rate of cancer for men is lung cancer, which is 19.4 per 100,000 population with an average death rate of 10.9 per 100,000 population, while the highest incidence rate of cancer for women is breast cancer, which is 42.1 per 100,000 population with an average 17 deaths per 100,000 population (Kemenkes RI, 2019).

Cancer treatment methods are still being developed. There are several types of local treatment such as surgery and radiation therapy, and systemic treatment such as chemotherapy, immunotherapy, or targeted therapy that can affect not only cancer cells but also the entire body (American Cancer Society, 2021). Understanding the causes, how they are formed, and especially the factors that promote or inhibit their growth required to solve the complex biological and medical problems. Currently, there are many successful results from cancer treatment. For example, over the past half century, the survival rate for childhood leukemia has increased from 10% to more than 80%. But the healing method (or extended remission) is difficult, and is only available to those who living in affluent countries. There is nothing like "medicine" for cancer, and much remains for medical researchers to do (Haussman, 2019).

The cancer treatment ideally can stop the growth of cancer cells with a selective toxic effect on the targeted cancer cells without damaging other normal tissues. However, most of today's anticancer drugs are still potentially toxic to normal tissues. The condition can cause harmful side effects to the body. Therefore, the discovery of anti-cancer drugs that have more positive effects with low toxicity is being developed. Many potential phytopharmaceuticals have been investigated to find chemical elements in plants that are beneficial for treatment (Wahyuningsih et al., 2013). The development of herbal studies using *in vitro* and *in vivo* methods has

shown the beneficial effects of medicinal plants and their bioactive compounds. The most common anti-cancer mechanism is by inducing apoptosis in neoplastic cells. Currently, more than 25% of drugs used over the last 20 years are derived directly from plants. According to WHO, 80 percent of the world's population has benefited from traditional medicine (Aryan, 2018). Nowadays, the efforts to develop new anti-cancer medicines and particularly novel drug delivery systems should be done. It is important to improve the pharmacodynamics and bioavailability of drugs and to deliver specific drug concentration to cancer cells so it can produce minimum cytotoxic effects on normal cells (Babazadeh et al., 2018).

Tithonia diversifolia CHARACTERIZATION

The Asteraceae family is known for its therapeutic effect such as antihelmintic, anti-inflammatory, astringent, antispasmodics, cholesteric, antioxidant, anti-hemorrhagic, antimicrobial, diuretics, and analgesics. One type of the species from this family is *Tithonia diversifolia* (Farias et al., 2019). *T. diversifolia* can be described as a flowering shrub-like species, growing up to over 2–3 m high. The flowers are 5–15 cm wide and shaped like a daisy. The leaves (15–30 cm long) can be described as sub-ovale, petiolate, 3- to 7-lobed delicately hairy, and alternately to oppositely arranged (Tagne at al., 2018). *T. diversifolia* (Hemsl.) A. Gray (Mexican sunflower) originally from Mexico and Central America, but is now widely known in various parts of the world as an ornamental plant and a plant used for medicine. There are more than 150 types of secondary metabolites isolated from *T. diversifolia*, including the sesquiterpene lactones thyrotundin, tagitinin A, and tagitinin C (Miranda et al., 2015). *T. diversifolia* can be found in tropical and subtropical climes. Traditionally, all parts of the plant especially the leaves, are used by people to treat wounds, musculoskeletal disorders, abscesses, dermatological conditions, stomach pains, oral treatment for diabetes, malaria, fever, hepatitis and infectious diseases. Some studies showed the heterogenous evidence from *in vitro* and *in vivo* research that supporting most of the traditional therapeutic claims. *T. diversifolia* can grow up for all year-round and used fresh or dry as traditional remedies in several cultures. All parts of the plant are useful include the leaves, flowers, stems and roots are mentioned in some ethnobotanical reports (Tagne et al., 2018).

The studies on phytochemical screening not only showed the presence of metabolites phenolics, flavonoids and tannins but also absence of alkaloids and saponins in *T. diversifolia* ethanolic flower extract. Phenolics are well known for their antioxidants and antimicrobial properties. This plays a role in prevention activities of phenolic against infection and degenerative disease, inflammation and allergies through antioxidants, antimicrobial and protein or enzyme neutralization or modulation mechanism. The examples of phenolic compounds are flavonoids and tannins. Flavonoids have many beneficial health effect and physiological properties. Flavonoids have antioxidant effects, anti-inflammatory, anti-mutagenic, anti-carcinogenic property and as a modulator to the function of cellular enzymes. It is also have a great potensial inhibitors of xanthine oxidase, cyclooxygenase, lipoxygenase and phosphoinositide 3-kinase. Tannins has antioxidant, anticarcinogenic and antimutagenic potential. Tannins are also known to accelerate blood clotting, lower blood pressure, lower serum lipid levels and modulation of immunoresponse. Terpenoids has an antibiotic, chemopreventive and therapeutic effects on cancer. Terpenoids have been developed in the prevention, inhibition and therapy of several diseases including cancer, antivirals, antimicrobial, anti-allergic, antispasmodic, antidiabetic, anti-inflammatory, anticancer and immuno-modulatory properties (Gaule Ang et al., 2019).

Farias et al, in 2019 also evaluate the composition, antioxidant, cytotoxic and microbiological activity of the essential oil of the leaves of the species *T. diversifolia.* The main constituent they was found are α-pinene (9.9%), Limonene (5.40%), (Z)-β-ocimene (4.02%), p-cymen-8-ol (3.0%), Piperitone (11.72%), (E)-nerolidol (3.78%) and Spathulenol (10.8%). The activity of antioxidant from thee essential oil extracted from the leaves of *T. diversifolia* can be observed from the significant result with $p < 0.0001$ at the concentration of 5 $mgmL^{-1}$ with %AA 54.6 ± 0.06. The minimum inhibitory concentration (IC50) was 4.30 $mgmL^{-1}$, with a strong correlation coeffificient (R2) of 0.9965. Although *T. diversifolia* has good antioxidant activity, its IC value still lower than ascorbic acid (Farias et al., 2019).

ANTI-CANCER POTENTIAL

Activity-guided fractionation technique of an ethyl acetate extract from *T. diversifolia*, using an antiproliferative bioassay against human colon cancer cells (Col2), resulted in the isolation of three new sesquiterpenoids, 2R-hydroxytirotundin (1), tithofolinolide (2), and 3R-acetoxydiversifolol. (3), together with eight known sesquiterpene lactones, 3'-acetoxy-8'-isobutyryloxyreynosine (4), tagitinin C (5), 1',2Repoxytagitinin C (6), 4R,10R-dihydroxy-3-oxo-8'-isobutyryloxyguaia -11(13)-en-12,6R-olide (7), 3R-acetoxy4R-hydroxy-11(13)-eudesmen-12-oic acid methyl ester, 17,20-dihydroxygeranylnerol, tagitinin A, and thyrotundine. Measurement of antiproliferative activity in Col2 cells and induction of cellular differentiation in human promyelocytic leukemia (HL-60) cells were carried out to determine the potential of these isolates as cancer chemopreventive agents. In this

study, a culture test was used on rat breast organs that had been induced by 7,12-dimethylbenz[a]anthracene and the selected compound was tested for its ability to inhibit preneoplastic lesions. Isolates tagitinin C and 1',2 Repoxytagitinin C showed significant antiproliferative activity. Isolat tithofolinolide, 3'-acetoxy-8'-isobutyryloxyreynosine, and 4R,10R-dihydroxy-3-oxo-8'-isobutyryloxyguaia -11(13)-en-12,6R-olide induced cellular differentiation of HL-60. The isolate 3'-acetoxy-8'-isobutyryloxyreynosine significantly inhibited (63.0% at 10 μgmL^{-1}) lesion formation in rat breast organ culture assays. Among these isolates there were several sesquiterpenoids and flavonoids that showed anti-human pro myelocytic leukemia (HL-60) activity and Tagitinin C was the main sesquiterpenoid that had been identified (Gu et al., 2002).

The methanol extract of *Tithonia diversifolia* showed antiproliferative activity against glioblastoma cells U373, with an IC_{50} value of 59.2 ±3.7 μgmL^{-1}, while tagitinin C showed an IC_{50} value of 6.1 ±0.1 μgmL^{-1}. Based on flow cytometric analysis and pan-caspase inhibitory activity, the anti-glioblastoma effect was independent of apoptosis and it was concluded that tagitinin C induces U373 cell death through autophagy under certain conditions. Autophagy is a transport pathway to all eukaryotic cells. Cells degrade part of their own intracellular constituents, including cytoplasm and organelles. The first process for autophagi mechanism is the formation of a double membrane bound vacuole from endoplasmic reticulum. The autophagosomes receive hydrolases to form an autolysosome. This study also found that human glioblatoma U373 cell death induced by tagitinin C was apoptosis-independent and induced autophagosome (Lee at al., 2011).

Another study showed the activity of the extract *T. diversifolia* in inhibiting the viability of keloid fibroblasts. Antifibrotic activity of the ethanolic extract of *T. diversifolia* on keloid fibroblast cell proliferation and collagen accumulation was expressed by the IC_{50} value using probit regression analysis. The percentage of inhibition of keloid fibroblast proliferation increases gradually with the given dose extract of *T. diversifolia* with a very strong correlation ($r = 0.838$; $p = 0.000$ in 72 hours; $r = 0.924$; $p = 0.000$ in 120 hours of incubation) with the lowest IC_{50} value of 3.624 μgmL^{-1} at an incubation time of 120 hours (Wahyuningsih et al., 2015). Furthermore, Ranti et al, in 2018 tested the cytotoxic activity of tagitinin C on keloid fibroblast cells with IC_{50} values of 0.122 μgmL^{-1} (72 hours incubation) and 0.039 μgmL^{-1} (120 hours) which affected the deposition of 53.1% of keloid collagen (72 hours) and 44.3% (120h). The selectivity index of tagitinin C in normal fibroblasts (NF) was 287 (72 hours incubation) and 791 for (120 hours incubation). Based on these results, it was concluded that the ability of tagitinin C to inhibit keloid fibrosis viability and reduce keloid collagen deposition was consistent with the concentration and incubation time. The process of collagen synthesis happened when the fibroblast got a stimulus from TGF-β that was bound to TGF-β receptors on the fibroblasts membrane. When an injury occured, the TGF-β will be separated from the binding protein give respond to mechanical trauma system. However, there have not been many studies on the inhibitory activity of TGF-β by *T. diversifolia* compound. But, another study have found that the sesquiterpene lactones is known to inhibit the keloid fibroblasts proliferation, inhibit TGF-β1, and inhibit collagen synthesis of keloid through the NF-κB (Ranti et al., 2018).

The next study was carried out by Wahyuningsih, et al in 2019 to determine the antifibrocytic mechanism of *T. diversifolia* extract. The study showed that the ethanolic extract of *T. diversifolia* at concentrations of 20 gmL^{-1}, 10 gmL^{-1}, and 5 gmL^{-1} for 24 hours in keloid fibroblast culture showed slower migration activity compared to untreated keloid fibroblast culture ($p<0$, 05). The study also showed that the expression of TGF-β1 and VEGF in the treatment group given the ethanolic extract of *T. diversifolia* was significantly lower than untreated keloid fibroblasts ($p<0.05$). From those result, it can be concluded that the ethanolic extract of *T. diversifolia* can inhibit fibroblast migration activity and reduce the expression of VEGF and TGF-β1 in keloid fibroblasts. This mechanism further strengthens that *T. diversifolia* has great potential to be used as a keloid therapy (Wahyuningsih et al., 2019).

Traditionally, *T. diversifolia* have been used to treat chronic hepatitis and liver diseases in Chinese herbal medicine. Further researh must be done to explore whether *T. diversifolia* can be used to treat hepatoma. (Lu et al., 2017). The study of the cytotoxic activity of the methanol extract of the leaves of *T. diversifolia* and tagitinin C against human hepatoma cells (Hep-G2) showed positive results. The test results with the MTT assay showed IC_{50} values 40.0 ± 2.0 and 2.0 ± 0.1 μgmL^{-1}. This compound induces an increase population in the sub-G1, exhibited S phase arrest and results in the activation of caspase 3 and caspase 8 which confirms the data that the antiproliferative effect of this compound is through caspase-dependent apoptotic activity. Tagitinin C caused membrane blebbing, cell shrinkage, nuclear fragmentation, and chromatin condensation in hepatoma cells. Caspases, have an essential role in the regulation and the execution of apoptotic cell death. Caspase 3 well known as a key effector caspase in the apoptosis pathway, amplifying the signal from initiator caspases (such as caspase 8). This study show that tagitinin C induced apoptosis done by the activation of the caspase cascade (Liao at al., 2012). A follow-up study was conducted by Lu in 2017 to showed another cytotoxic effect of *T. diversifolia* on Hep G2 cells. The results of the study showed that the induction of apoptosis such as DNA fragmentation could increase the level of apoptosis and apoptosis of the sub-G1 population in the treatment group that given the acetate extract leaves of *T.*

diversifolia. In addition, the acetate extract of the leaves of *T. diversifolia* was also increase the expression of the proapoptotic protein Bax Bcl-2. Based on this study, it prove the cytotoxicity induced by ethyl acetate extracts of *T. diversifolia* leaves (Td-L-EA) in human HepG2 hepatoma cells that strengthen ethnopharmocologic use of *T. diversifolia* (Lu at., 2017).

The cytotoxic effect of *T. diversifolia* leaf extract on RAW264.7 cells and human mononuclear peripheral blood cells (PBMC) by mitochondrial respiration method was also studied. The results of the study showed that the IC_{50} concentration in PBMC proliferation was 4.42 μgmL^{-1} and in RAW264.7 cells the IC_{50} value was 11.63 μgmL^{-1}. The study demonstrated an immunomodulating effect by the leaf extract of *T. diversifolia*, which was produced the inhibition of phytohemagglutinin-M-induced PBMC proliferation and LPS-induced nitric oxide production in RAW264.7 macrophages. The hydrogen peroxide are able to induce cell death under stress conditions through apoptosis, but the anti-oxidant compound can protect this condition. The data showed that *T. diversifolia* aqueous extract showed an anti-oxidative capacity equivalent of (241.04 ± 11.93 mmol $Troloxg^{-1}$) from ABTS assay and (94.89 ± 2.69 mmol $Troloxg^{-1}$) from DPPH assay, respectively. The anti-oxidative capacity of *T. diversifolia* aqueous extract was equivalent to (32.62 ± 1.87) and (20.99 ± 2.79) mg $NACg^{-1}$ in the ABTS and DPPH assays, when NAC was used as the standard compound. However, the calculated concentration was out of the non-cytotoxic range, which should be lower than 30 mg/mL. Thus, this calculated concentration based on the antioxidant capacity could not be used in practice in the hydrogen peroxide-induced cell death model.

It could be conclude that the effects of the immunomodulation caused by the aqueous extract from *T. diversifolia.* The mechanism can desccribe such as anti-PHA-M-induced PBMCs proliferation and antiLPS-induced nitric oxide production from macrophages in the range of non-cytotoxic concentrations (Hiransai et al., 2016).

CONCLUSION

Based on the literature study, it is found that some isolate from the extract of *Tithonia diversifolia* (Hemsley) A. Gray) have the anticancer activity in several different types of cancer cells. The active substances contained in the leaves of the *T. diversifolia*. The compound material which has been known to become an anticancer alternative therapy is tagitinin C. The extract of the leaves *T. diversifolia* has several anticancer mechanisms related to antifibrotic activity and the induction of apoptosis. Further research can be develop to find out another potential compound from the *T. diversifolia* extract. We hope there will be a new chance to find better alternative therapy for cancer.

Conflict of interest: The author declares that there are no conflicts of interest concerning the publication of this article.

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