



REVIEW ARTICLE

Anticancer Activity of *Typhonium flagelliforme*: A Systematic Review

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ABSTRACT

Cancer is a global health issue that can affect anyone. Cancer is treated conventionally with surgery, chemotherapy, radiotherapy, and hormone therapy. However, the high cost of conventional treatments is a burden for cancer patients. Therefore, many cancer patients seek cheaper yet effective alternative treatments. *Typhonium flagelliforme* is a taro-like plant that can be found across Indonesia. Numerous researches on the anticancer effect of *T. flagelliforme* have been conducted. However, a systematic review on the anticancer property of *T. flagelliforme* is still lacking. Therefore, this review aimed to systematically evaluate the scientific evidence for the anticancer activities in *T. flagelliforme*. Five databases were used as the search engine using the designated search terms, and studies were selected based on the inclusion criteria. The anticancer evaluation in 30 studies selected was conducted in leukemia, lymphoma, breast, oral, cervical, lung, liver, colon, and squamous cell carcinoma. The result showed that *T. flagelliforme* could inhibit cancer cell proliferation, with most of the IC₅₀ being less than 200 µg/mL. *T. flagelliforme* induced an increase of caspase-3 and -9 and a decrease in the anti-apoptotic Bcl-2 protein expression. In addition, the expression of p21 protein was increased after treatment of *T. flagelliforme* extract. In contrast, the tyrosine kinase, Ki67, HER2/neu, telomerase, and COX-2 expressions were decreased, implying that *T. flagelliforme* could inhibit tumor growth and development. Lastly, *T. flagelliforme* is also capable of reducing the possibility of cancer cell invasion. Findings suggest that *T. flagelliforme* has the potential to be further developed for cancer treatment.

Keywords: *Typhonium flagelliforme*; Cancer; Systematic review

HIGHLIGHTS

- ❖ *T. flagelliforme* is an Indonesian taro-like plant that has anticancer properties.
- ❖ *T. flagelliforme* can inhibit cancer cell proliferation.
- ❖ *T. flagelliforme* increases pro-apoptotic and decreases anti-apoptotic protein expression.
- ❖ *T. flagelliforme* can reduce cancer cell invasion.

INTRODUCTION

Cancer is a worldwide known disease that affects anyone regardless of gender or age. It is a disease state where the cells in one part of the body are continuously and abnormally growing that can then invade other nearby healthy cells. Cancer has become one of the most common causes of morbidity and mortality (Stewart & Wild, 2014). It was estimated that there were 19.3 million new cancer cases worldwide in 2020 and predicted to reach 28.4 million by the year 2040, while there was approximately 10 million cancer mortality in 2020 and predicted to keep increasing as the number of cases increase (Sung et al., 2021). In Indonesia, the data on cancer prevalence from Basic Health Research 2018 was 21,600 out of 1.2 million

people, about 1.8 in 100 people (Kementerian Kesehatan RI, 2019). World Health Organization (WHO) (2020) reported that the most common cancers in Indonesia are breast, cervical, lung, colorectum, and liver cancer. These types of cancers also have a high mortality rate in Indonesia. Lifestyle, environmental, and behavioral exposure, such as smoking and drinking, are risk factors for most cancers.

There are many types of treatment available for cancer patients. The aim is to cure the disease, prolong patients' life, and improve the quality of life for the patients. Although the treatment for cancer might differ from one type of cancer to another, surgery, radiotherapy, chemotherapy, and hormone therapy are most commonly done for cancer patients (World Health Organisation, 2008). However, the cost of those therapies is very high. According to ASEAN Cost in Oncology (ACTION) study, more than 75 percent of cancer patients in South East Asia experience financial burden twelve months after the patient is diagnosed with cancer (Kimman et al., 2015). Furthermore, some reasons such as: 1) several failures yet expensive cases of conventional cancer therapy, 2) several successful cases of herbal medicine therapy, and 3) a variety of herbal medicines available in Indonesia, are what causes cancer patients to seek herbal therapy (Hasanah & Widowati, 2016). Hence, a cheaper and more effective alternative for cancer treatment is needed; and herbal medicine, especially *Typhonium flagelliforme*, is one of the potential treatments to be investigated for cancer.

Keladi Tikus or Rodent Tuber in English (*Typhonium flagelliforme*) is a taro-like plant that can be found in Malaysia, South Korea, and Indonesia. In Indonesia, *T. flagelliforme* is spread along Java island, Kalimantan, Sumatera, and Papua (Widowati & Mudahar, 2009). It contains alkaloids, saponins, steroids, glycosides, hexadecanoic acid, and oleic acid (Kristina, 2007; Lai, Mas, Nair, Mansor, & Navaratnam, 2010). There has been a lot of research about *T. flagelliforme* in various cancer cells that showed *T. flagelliforme* possessed anticancer activity, acted as an antioxidant that potentially inhibited the proliferation of tumor cells (Setiawati, Immanuel, & Utami, 2016), and triggered apoptosis of the cell (Da'i, Fiveri, & Meiyanto, 2007). Previous studies on the anticancer activity of *T. flagelliforme* show that *T. flagelliforme* is a potential candidate as an alternative medicine for Indonesian cancer patients. Therefore, this review aims to systematically evaluate the scientific evidence for anticancer activities of *T. flagelliforme*.

MATERIAL AND METHODS

This systematic review was done following the PRISMA Guideline (Page et al., 2021).

Search strategy

Science Direct, PubMed, Google Scholar, Garuda, and Cochrane Library databases were used to search the studies regarding the investigation of the effects of *Typhonium flagelliforme* on cancer treatment using the search terms ("*Typhonium flagelliforme*" OR "rodent tuber" OR "*keladi tikus*") AND ("cancer" OR "neoplasm" OR "neoplasms" OR "tumor" or "tumors" OR "kanker").

Eligibility criteria

The studies were independently screened and selected by two investigators (AC and HH) based on the inclusion and exclusion criteria. The inclusion criteria are: 1) papers that use cancer cells or animal cancer models as the investigating condition, 2) full-text manuscript of the study is accessible (i.e., original research articles and thesis), and 3) the outcomes included are the hallmarks of cancer (i.e., cell proliferation, apoptotic activity, invasion activity, angiogenesis, and cancer-related gene mutation level). The exclusion criteria are: 1) studies that are not in English or *Bahasa Indonesia*, 2) the main treatment of the studies is not *Typhonium flagelliforme*, 3) a combination of *T. flagelliforme* with other herbs or drugs, 4) the condition being investigated is not cancer, and 5) abstract only or inaccessible full-text manuscripts. Any disagreement on the study's selection was resolved through discussion between the investigators.

Data extraction

Data extracted from the studies include the author, publication year, study design, cell type or animal model used in the study, treatment concentration or dosage regimen, treatment route of administration, parameter observed in the study, and the result of the study.

Quality assessment

To ensure the quality of the studies used for this review, a quality assessment was conducted independently by two investigators (AC and HH). A quality assessment tool, ToxRTool, was used for both *in vivo* and *in vitro* studies. The evaluated parameters include the identification of the test substance, the test system characterization, the study design description, the study results documentation, and the plausibility of the study design and result (Schneider et al., 2009).

RESULTS

A total of 944 studies from 1992 up to June 2020 were obtained from Science Direct, PubMed, Google Scholar, and Garuda. No study was found from the Cochrane Library search. The articles were checked for duplications using Mendeley, and a total of 722 studies were screened for their title and abstract for their eligibility based on the inclusion and exclusion criteria. As shown in Figure 1, full-text screening was conducted for 90 studies which resulted in a total of 30 eligible studies included in this systematic review. Out of 30 studies, 27 studies are *in vitro* studies, 1 study contains both *in vitro* and *in vivo* studies, while 2 studies are *in vivo* studies.

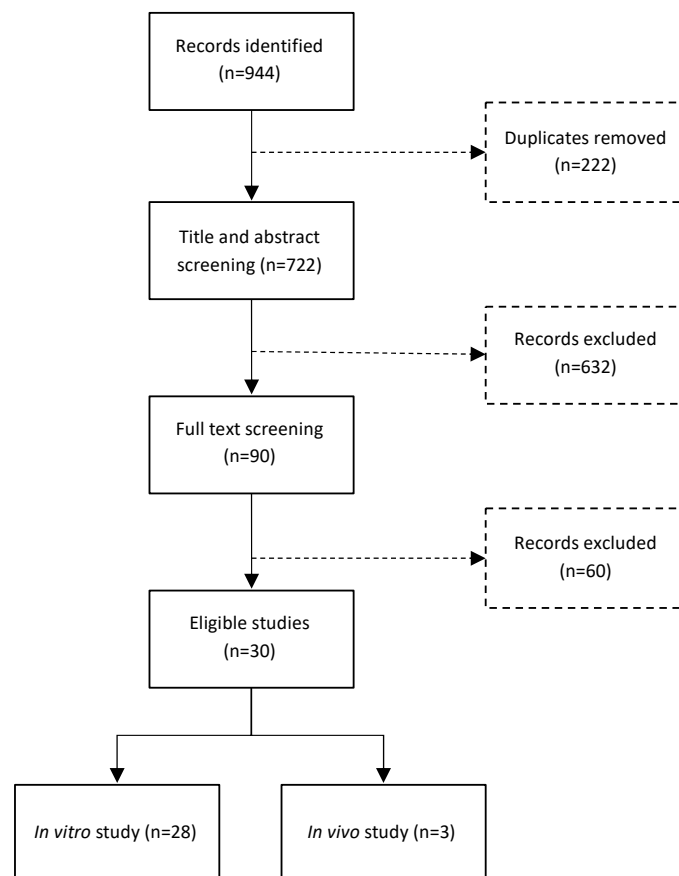


Figure 1. Flow diagram of studies selection

A quality assessment conducted using ToxRTool deemed 28 articles are reliable without restriction, while 2 *in vitro* studies are reliable with restriction. The reliability of the restriction quality assessment results was mainly due to no origin of the plant and cells reported in the articles and no mention of replication and statistical significance.

The studies included in this review were conducted in various cancer cells as well as cancer animal models, as detailed in Table 1. The types of cancer investigated were breast cancer, leukemia, lymphoma, oral cancer, cervical cancer, lung cancer, liver cancer, colon cancer, and squamous cell carcinoma.

Table 1. Data extraction

| Author, publication year | Study design | Cell type/animal model | Treatment | Treatment concentration/dose | Treatment duration | Parameter | Study results |
|--|-----------------|---|---|---|----------------------|---|---|
| Chodidjah, Susanto, & Sarijadi, 2013 | <i>In vivo</i> | Mice with mammary adenocarcinoma | Oral TF (tubers) ethanol extract | 200, 400, 800 mg/kg BW | 30 days | Tyrosine kinase level; Ki67 expression | Reduced tyrosine kinase level; reduced Ki67 expression (p<0.05) |
| Chodidjah, Nasihun, Widayati, & Goenarwo, 2014 | <i>In vivo</i> | Mice with breast cancer | Oral TF (tubers) ethanol extract | 8 and 16 mg | 25 days | Bcl-2 expression; HER2/neu expression; breast cancer volume | Reduced Bcl-2 expression; reduced HER2/neu expression (p<0.05); similar breast cancer volume (p>0.05) |
| Mohan et al., 2010 | <i>In vivo</i> | Mice injected with WEHI-3 murine leukemia cells | Oral TF (whole plant) DCM extract | 200, 400, 800 mg/kg BW | 28 days | Leukocyte level; histopathology of liver and spleen; apoptotic activity in the spleen | Reduced immature cell in peripheral blood; decreased spleen and liver tumor size; increased apoptotic cells in the spleen (p<0.05) |
| | <i>In vitro</i> | WEHI-3 murine leukemia cells | TF (whole plant) hexane, ethyl acetate, DCM, methanol extract | Various concentration (cytotoxic activity IC ₅₀ (apoptotic activity study) | 68h; 24, 48, and 72h | Cytotoxic activity; apoptotic activity | DCM showed lowest IC ₅₀ = 24 µg/mL in cytotoxic activity; IC ₅₀ of DCM extract induced apoptosis (DNA fragmentation, blebbing, nuclear chromatin condensation) (p<0.05) |

| Author, publication year | Study design | Cell type/animal model | Treatment | Treatment concentration/dose | Treatment duration | Parameter | Study results |
|--|-----------------|--|--|--|--|---|--|
| Choo, Chan, Takeya, & Itokawa, 2001 | <i>In vitro</i> | P388 murine leukemia cell line | TF (stem and leaves or roots and tubers) hexane, chloroform, butanol extract, methanol residue | Various concentration | 48h | Cytotoxic activity | Chloroform IC ₅₀ = 6 and 8 µg/mL (roots and tubers and stems and leaves, respectively), hexane IC ₅₀ = 15 and 65 µg/mL (roots and tubers and stems and leaves, respectively), butanol and methanol residue IC ₅₀ >100 µg/mL |
| Choo, Chan, Sam, Hitotsuyana gi, & Takeya, 2001 | <i>In vitro</i> | P388 murine leukemia cell line | Fractions of TF (tubers) hexane extract | Various concentration | 48h | Cytotoxic activity | Hexane IC ₅₀ = 15 µg/mL; fractions show cytotoxicity with IC ₅₀ <15 µg/mL |
| S Mohan, Bustamam, Ibrahim, Al-Zubairi, & Aspollah, 2008 | <i>In vitro</i> | CEM-ss human T4-lymphoblastoid cell line | TF (leaves and tubers) hexane, ethyl acetate, DCM, methanol extract | 3.375, 6.25, 12.5, 25, 50, 100 µg/mL | 72h | Cell proliferation; morphological study | DCM, ethyl acetate extract IC ₅₀ <11 µg/mL, hexane and methanol extract IC ₅₀ >50 µg/mL; morphology of cells treated with DCM and ethyl acetate extract showed cell shrinkage, membrane blebbing, chromatin condensation, and DNA fragmentation. |
| Mohan, Abdul, Abdelwahab, Al-Zubairi, Sukari, et al., 2010 | <i>In vitro</i> | CEM-ss human T4-lymphoblastoid cell line | Fractions of TF (whole plant) DCM Extract | Various concentrations (Cell proliferation study); 6 µg/mL; 3, 10, 20 µg/mL; 3 µg/mL | 68h; 6, 12, 24, 48h; 72h; 48h; 12, 24, 48, 72h | Cell proliferation; apoptotic activity; caspase activity; apoptosis pathway; cell cycle | IC ₅₀ = 3 µg/mL; induced apoptosis (loss of membrane integrity, DNA fragmentation); increased caspase-3 and -9; mitochondria-dependent signaling pathway; cell arrest at G0/G1 phase |

| Author, publication year | Study design | Cell type/animal model | Treatment | Treatment concentration/dose | Treatment duration | Parameter | Study results |
|--|-----------------|--|--|---|-------------------------------|---|--|
| Mohan et al., 2011 | <i>In vitro</i> | CEM-ss human T4-lymphoblastoid cell line | Fractions of TF (whole plant) DCM extract | Various concentration (Cell proliferation study); IC ₅₀ of 3 µg/mL | 68h; 24, 48, 72h; 12, 24, 48h | Cell proliferation; apoptotic activity (morphology); apoptotic activity (ApoBrdU-TUNEL assay) | Fractions IC ₅₀ = 3 - >25 µg/mL; cell blebbing, nuclear chromatin condensation, cell shrinkage, apoptotic body formation; increased in DNA fragmentation (p<0.05) |
| Purwaningsih, Suciati, & Widayanti, 2017 | <i>In vitro</i> | Raji cell lines | TF (whole plant) ethanol extract | IC ₅₀ = 53.8 µg/mL; half of IC ₅₀ | 24h | Telomerase expression | Reduced telomerase expression |
| Harhari, Supriatno, & Medawati, 2011 | <i>In vitro</i> | SP-C1 human oral cancer cell line | TF (leaves) ethanol extract | 0, 25, 50, 75, 100, 125 µg/mL | 24, 48h | Cell proliferation | Reduced cell proliferation (p<0.05) |
| Ridlo, Supriatno, Medawati, & Rahmawati, 2012 | <i>In vitro</i> | SP-C1 human oral cancer cell line | TF (leaves) ethanol extract | 0, 25, 75, 100, 125 mg/mL | 48h | Apoptotic activity | Dose-dependent increased in apoptotic activity (p<0.05) |
| Zakiyana, Supriatno, & Medawati, 2010 | <i>In vitro</i> | SP-C1 human oral cancer cell line | TF (leaves) ethanol extract | 0, 25, 50, 75, 100, 125 µg/mL | 24h | Invasion activity | Reduced cell invasion (p<0.05) |
| Widowati & Mudahar, 2009 | <i>In vitro</i> | MCF-7 breast cancer cell line | TF (tubers) ethanol extract | 50, 75, 100, 125, 150 µg/mL | 24h | Cell proliferation | Reduced cell proliferation (IC ₅₀ = 89.16 µg/mL) |
| Sianipar, Assidqi, Purnamaningsih, & Herlina, 2019 | <i>In vitro</i> | MCF-7 breast cancer cell line | TF (tubers) ethanol extract | 62.5, 125, 250, 500, 1000 µg/mL | 24h | Cell proliferation | Reduced cell proliferation (IC ₅₀ = 12.482 and 7.043 µg/mL of mutant plants by gamma-ray irradiation; IC ₅₀ = 19.113 µg/mL of wild type plant) |
| Putra, Tjahyono, & Winarto, 2012 | <i>In vitro</i> | MCF-7 breast cancer cell line | TF (tubers or leaves) DCM, ethanol, hexane extract | 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/mL | 68h | Cell proliferation | Reduced cell proliferation (p<0.05), DCM extract showed the best results with IC ₅₀ = 63.08 |

| Author, publication year | Study design | Cell type/animal model | Treatment | Treatment concentration/dose | Treatment duration | Parameter | Study results | |
|---|-----------------|---------------------------------|--------------|---|---|-----------------|--|--|
| | | | | | | | and 68.65 µg/mL of tuber and leaves extract, respectively | |
| Nobakht, Kadir, Stanslas, & Charng, 2014 | <i>In vitro</i> | MCF-7 breast cancer cell line | human cancer | TF (whole plant) methanol and DCM mixed extract | 0.001, 0.01, 0.1, 1 mg/mL | 96h | Cytotoxic activity | Reduced cell proliferation IC ₅₀ >100 µg/mL (in vitro), IC ₅₀ < 100 µg/mL (ex vitro) |
| Alfarabi et al., 2015 | <i>In vitro</i> | MCF-7 breast cancer cell line | human cancer | TF (tuber NaCl extract (crude protein extract) | 6.25, 12.5, 25, 50, 100, 200 ppm | 24h | Cell proliferation | Decreased cell proliferation, IC ₅₀ = 90.78 ppm |
| Putra, Tjahjono, & Winarto, 2011 | <i>In vitro</i> | MCF-7 breast cancer cell line | human cancer | TF (tuber or leaves) DCM extract | 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/mL; IC ₅₀ | 48h; 5, 10, 20h | Cell proliferation; apoptotic activity; p21, and caspase-3 expression; p21 and caspase-3 correlation | Reduced cell proliferation, IC ₅₀ = 63.08 and 68.65 µg/mL of tuber and leaves extract, respectively; DNA fragmentation, membrane blebbing, apoptotic body formation; increased p21 and caspase-3 expression; no significant correlation between p21 and caspase-3 |
| Farida, Martati, Musir, & Edward, 2010 | <i>In vitro</i> | T47D breast carcinoma cell line | human cell | TF (leaves) methanol, n-hexane extract | 10, 25, 50, 100, 250, 500 µg/mL | 24h | Cell proliferation | n-hexane showed the best IC ₅₀ of 32.50 µg/mL |
| Nurrochmad, Lukitaningsih, & Meiyanto, 2011 | <i>In vitro</i> | T47D breast carcinoma cell line | human cell | TF ethanol extract* | 0, 10, 25, 50, 100, 250, 500, 1000 µg/mL | 48h; 24, 48h | Cell proliferation; apoptosis | Reduced cell proliferation at concentration higher than 250 µg/mL (IC ₅₀ = 632 µg/mL); induced apoptosis |

| Author, publication year | Study design | Cell type/animal model | Treatment | Treatment concentration/dose | Treatment duration | Parameter | Study results |
|------------------------------------|-----------------|--|---|--|--------------------|--|---|
| Nasir & Bohari, 2015 | <i>In vitro</i> | MDA-MB-231 breast cancer cell line | TF (leaves) methanol extract | 0.0078, 0.0313, 0.125, 0.25, 0.50, 1.0 mg/mL | 72h | Cell proliferation; cell morphology | Decreased cell proliferation (IC_{50} = 0.11 mg/mL); cell shrinkage ($p < 0.05$) |
| Da'i et al., 2007 | <i>In vitro</i> | HeLa human cervical cancer cell line | TF (leaves) ethanol, ethyl acetate. Chloroform extracts | 5, 10, 25, 50, 125, 250 μ g/mL | 24h | Cytotoxic activity | Ethyl acetate extract possessed higher cytotoxic activity (IC_{50} = 147.77 μ g/mL) than chloroform (IC_{50} = 903.44 μ g/mL) and ethanol (IC_{50} could not be specified) |
| Lai et al., 2008 | <i>In vitro</i> | NCI-H23 human lung cancer cell line | TF (whole plant) hexane, DCM, methanol extracts | 0.78-100 μ g/mL | 72h; 24, 48, 72h | Cell proliferation; apoptotic activity | DCM IC_{50} = 15.43 μ g/mL, hexane IC_{50} = 53.89 μ g/mL, methanol IC_{50} >100 μ g/mL; DCM showed apoptotic activity: cytoplasmic shrinkage, membrane blebbing, DNA fragmentation |
| Setiawati, Immanuel, & Utami, 2016 | <i>In vitro</i> | WiDr human colon cancer cell line | TF (leaves) ethyl acetate extract | Various concentrations (Cell proliferation assay); 100 μ g/mL (apoptosis and COX-2 inhibition assay) | 24h | Cell proliferation; apoptotic activity; COX-2 expression | Reduced cell proliferation IC_{50} = 70 μ g/mL; induced apoptosis; inhibit COX-2 expression ($p < 0.05$) |
| Chan, Koh, & Tengku-Muhammad, 2005 | <i>In vitro</i> | T47D human breast cancer cell lines, NCI-H23 human non-small cell lung cancer cell lines, HepG2 liver cancer cell line | TF hexane, chloroform, butanol extract* | 1, 10, 25, 50, 75, 100, 200 μ g/mL | 72h | Cytotoxic activity | All extracts showed cytotoxic activity on NCI-H23 IC_{50} <20 μ g/mL, on T47D IC_{50} <50 μ g/mL, no significant cytotoxic activity on HepG2 (IC_{50} >100 μ g/mL) |

| Author, publication year | Study design | Cell type/animal model | Treatment | Treatment concentration/dose | Treatment duration | Parameter | Study results |
|--|-----------------|--|---|--|--------------------|-------------------------------|---|
| Purwaningsih, Widayanti, & Suciati, 2014 | <i>In vitro</i> | HeLa human cervical cancer cell line; MCF-7 human breast cancer cell line | TF (whole plant) ethanol extract | 3.9, 7.81, 15.625, 31.25, 62.5, 125, 250, 500 µg/mL | 48h | Cell proliferation | Reduced cell proliferation with IC ₅₀ = 30.19 µg/mL (HeLa); IC ₅₀ = 5.59 µg/mL (MCF-7) |
| Purwaningsih, Suciati, & Widayanti, 2016 | <i>In vitro</i> | HeLa human cervical cancer cell line; T47D human breast cancer cell line | TF (whole plant) ethanol extract | IC ₅₀ = 30.19 µg/mL; half of IC ₅₀ | 24h | Telomerase expression | Reduced telomerase expression in both cell lines (p<0.05) |
| Lai et al., 2010 | <i>In vitro</i> | NCI-H23 human non-small cell lung cancer cell line; HS578T human breast carcinoma cell line | Fractions of TF (whole plant) DCM extract | 1.56, 3.13, 6.25, 12.5, 25, 50 µg/mL | 24h; 72h | Cell proliferation; apoptosis | Reduced cell proliferation IC ₅₀ = 2.7 µg/mL (NCI-H23), IC ₅₀ = 2.6 µg/mL (HS578T); induced apoptosis (cell shrinkage, cell blebbing) |
| Rostantinata et al., 2018 | <i>In vitro</i> | MCA-B1 canine oral acanthomatous epulis cell line, MCM-B2 canine mammary gland benign mixed tumor cell line | TF (leaves) ethanol extract | 0, 20, 40, 60, 80, 100, 120 ppm | 3-4 days | Cell proliferation | Reduced cell proliferation |
| Priosoeryanto et al., 2020 | <i>In vitro</i> | CSCC canine squamous cell carcinoma cell line, FSCC feline squamous cell carcinoma cell line, MCM-IPB-B3 canine mammary gland benign mixed tumor cell line | TF (leaves) ethanol extract | 120 ppm | 4 days | Cell proliferation | Low activity in reducing cell proliferation |

Abbreviation: DCM, dichloromethane; h, hour; IC₅₀, concentration resulted in 50% inhibition; TF, *T. flagelliforme*.

* No information on plant parts used for extraction in the study.

Breast cancer

Breast cancer was the most used cancer cell line, and animal models were found to investigate the effect of *T. flagelliforme* on cancer. In total, there were 17 studies regarding the anticancer effect of *T. flagelliforme* extract in breast cancer cells. In addition, two studies were conducted *in vivo*, and 15 studies conducted were *in vitro* using several breast cancer cell lines such as MCF-7 human breast cancer cell lines, T47D human breast cancer cell lines, HS578T human breast cancer cell lines, MCM-B2 canine mammary gland

benign mixed tumor cell lines, and MCM-IPB-B3 canine mammary gland benign mixed tumor cell lines (Alfarabi et al., 2015; Chan et al., 2005; Chodidjah et al., 2013, 2014; Farida et al., 2010; Lai et al., 2010; Nasir & Bohari, 2015; Nobakht et al., 2014; Nurrochmad et al., 2011; Priosoeryanto et al., 2020; Purwaningsih et al., 2016, 2014; Putra et al., 2012; Rostantinata et al., 2018; Sianipar et al., 2019; Widowati & Mudahar, 2009).

Studies on cell proliferation found that *T. flagelliforme* extract could inhibit breast cancer cell proliferation with IC₅₀ ranging from 2.6 to above 100 µg/mL in various breast cancer cell lines (Alfarabi et al., 2015; Chan et al., 2005; Farida et al., 2010; Nasir & Bohari, 2015; Nobakht et al., 2014; Nurrochmad et al., 2011; Purwaningsih et al., 2014; Putra et al., 2011, 2012; Sianipar et al., 2019; Widowati & Mudahar, 2009). It was also found that treatment of *T. flagelliforme* extract on MCF-7 cells caused increased p21 and caspase-3 expression with no correlation between the two (Putra et al., 2011). Telomerase expression was also found to be reduced from 89% to 31% after treatment of *T. flagelliforme* extract on the T47D cell line (Purwaningsih et al., 2016). Several studies *in vitro* also found that *T. flagelliforme* extract caused cell apoptosis signified by DNA fragmentation, membrane blebbing, cell shrinkage, and formation of apoptotic bodies (Lai et al., 2010; Nasir & Bohari, 2015; Putra et al., 2011)

There were two *in vivo* studies conducted by Chodidjah et al. (2013, 2014). Both studies used mammary adenocarcinoma cells to be implanted in C3H mice and treated with *T. flagelliforme* tuber ethanol extract to study the tyrosine kinase expression, Ki67 expression, HER2/neu expression, Bcl-2 expression, and breast cancer volume. The tyrosine kinase expression in the negative control group was 22.13x10⁵ unit/mg; the lowest expression was observed in the 200 mg/kg extract BW-treated group at 9.5x10⁵ unit/mg (p<0.05). In contrast, the 400 and 800 mg/kg BW showed a minimal reduction of tyrosine kinase expression at 17.46x10⁵ unit/mg and 19.15x10⁵ unit/mg, respectively. The Ki67 expression in the control group was 90.83%, followed by 73.33%, 66.00%, and 21.66% for the group treated with 400, 800, and 200mg/kg BW of extract, respectively (Chodidjah et al., 2013). Treatment of *T. flagelliforme* extract showed a decrease in HER2/neu expression as the treatment dose increased (245.40% was in the control group, 235.50% in the 40 mg/mL group, and 51.60% in the 80 mg/mL group). It was also found that there was 114.40% Bcl-2 expression in the untreated group, 54.20% in the 40 mg/mL group, and 24.60% in the 80 mg/mL group. In contrast, the study found that the extract increased the breast cancer volume as the treatment concentration increased from 2563.86 mm³ to 3667.15 mm³ to 4392.33 mm³ (Chodidjah et al., 2014).

Leukemia

A total of 7 *in vitro* studies and 1 *in vivo* study investigated the effect of *T. flagelliforme* extract on leukemia cancer cell lines and animal models. The *in vitro* studies evaluated cytotoxic activity, apoptotic activity, caspase activity, and telomerase expression levels on WEHI-3, P388, CEM-ss, and Raji cell lines. It was found that *T. flagelliforme* extract had IC₅₀ ranging between 6 to above 100 µg/mL, with chloroform extract showing the lowest IC₅₀ while DCM extract showed the second-lowest IC₅₀ (Choo, Chan, Sam, et al., 2001; Choo, Chan, Takeya, et al., 2001; Mohan, Abdul, Abdelwahab, Al-Zubairi, Aspollah Sukari, et al., 2010; Mohan, Abdul, Abdelwahab, Al-Zubairi, Sukari, et al., 2010; Mohan et al., 2008, 2011; Purwaningsih et al., 2017). In addition, *T. flagelliforme* extract was able to induce apoptosis in leukemia cell lines. It was observed through DNA fragmentation, membrane blebbing, nuclear chromatin condensation, and apoptotic body formation, as well as cell shrinkage (Mohan, Abdul, Abdelwahab, Al-Zubairi, Aspollah Sukari, et al., 2010; Mohan, Abdul, Abdelwahab, Al-Zubairi, Sukari, et al., 2010; Mohan et al., 2008, 2011).

An increase in caspase-3 and -9 was found on CEM-ss cells treated with *T. flagelliforme* extract. In the immunoblot analysis, PARP (a substrate for caspase-3) was activated, and cytochrome c from mitochondria was released into the cytosol, which indicates the apoptosis by the extract in CEMss cells took place in the mitochondrial-dependent apoptotic pathway. During the apoptosis induction, it was shown that Bcl-2 decreased after cells were treated with the extract. This study also demonstrated that the cell cycle halted its progression significantly at the G0/G1 phase (p<0.05) (Mohan, Abdul, Abdelwahab, Al-Zubairi,

Sukari, et al., 2010). In addition, *T. flagelliforme* extract was also shown to be able to reduce telomerase expression in Raji cell lines treated with IC₅₀ and half of IC₅₀ of the extract (Purwaningsih et al., 2017).

In vivo study using BALB/c mice introduced with WEHI-3 cell showed that the number of peripheral granulocytes and monocytes in groups treated with *T. flagelliforme* extract and positive control was significantly lower than in untreated leukemic mice group and vehicle-only treatment groups. *T. flagelliforme* extract also significantly reduced the spleen tumor and liver size in a dose-dependent manner. TUNEL assay also confirmed that the spleen treated with *T. flagelliforme* extract increased the number of apoptotic cells (Mohan, Abdul, Abdelwahab, Al-Zubairi, Aspollah Sukari, et al., 2010).

Oral cancer

Three studies used *T. flagelliforme* leaves ethanol extract in SP-C1 human oral cancer to observe the cell proliferation activity, apoptotic activity, and invasion activity. One study by Harhari et al. (2011) suggested that the extract significantly decreased cell proliferation as the dose increased without any report on the IC₅₀ value. Ridlo et al. (2012) showed that the extract significantly increased apoptotic activity as the dose increased. Another study by Zakiyana et al. (2010) showed that the invasion activity decreased as the treatment concentration increased. The invasion study was done in the Boyden chamber, and after 24-hour of incubation, the invasion activity started to decrease at the concentration of 50 µg/mL (p<0.05). A study on canine oral cancer cell lines also showed that *T. flagelliforme* extract inhibited cell proliferation as the concentration increased (Rostantinata et al., 2018).

Cervical cancer

Da'i et al. (2007) compared the cytotoxic effectivity from the leaves of *T. flagelliforme* ethanol, ethyl acetate, and chloroform extract in the HeLa human cervical carcinoma cell line using an MTT assay. Results showed that ethyl acetate extract possessed the highest cytotoxic activity with the IC₅₀ of 147.77 µg/mL. Another cytotoxic activity study by Purwaningsih et al. (2014) using *T. flagelliforme* whole plant ethanol extract showed activity with IC₅₀ of 30.19 µg/mL. Treatment of *T. flagelliforme* whole plant ethanol extract using IC₅₀ of 30.19 µg/mL and half of IC₅₀ managed to reduce telomerase expression on HeLa cells from 81.67% to 22.33% and 11.00% with half IC₅₀ and IC₅₀ treatment, respectively (Purwaningsih et al., 2016).

Lung cancer

A total of 3 studies on lung cancer used the NCI-H23 non-small human cell lung carcinoma cell line. Lai et al. (2008) examined the cytotoxic effect from the whole plants of *T. flagelliforme* hexane, dichloromethane, and methanol extract for 72 hours, resulting in the IC₅₀ of 53.89, 15.43, and above 100 µg/mL, respectively. Fractions of hexane and DCM extracts showed the lowest IC₅₀ at 9.51 and 7.49 µg/mL, respectively. Apoptosis assay of a fraction of DCM extract showed that at hour-24, cytoplasmic shrinkage and membrane blebbing were observed. Cells were already broken into small apoptotic bodies at hour-48 with TUNEL assay confirmation which showed a DNA fragmentation event (Lai et al., 2008). Another study also showed that the fraction of *T. flagelliforme* DCM extract has a potent cytotoxic effect with IC₅₀ of 2.7 µg/mL in addition to causing membrane blebbing and cell shrinkage on NCI-H23 cells (Lai et al., 2010).

A study by Chan *et al.* (2005) using hexane, chloroform, and butanol extract of *in vitro* and field-grown plants to observe the cytotoxic activity of *T. flagelliforme* for 72 hours showed that all extracts from both *in vitro* and field-grown plants possessed IC₅₀ of less than 20 µg/mL, with smallest IC₅₀ value of 1.2 µg/mL from butanol field-grown plant extract.

Liver cancer

There was only one study conducted on liver cancer. HepG2 human liver cancer cells were used to observe the cytotoxic activity of *T. flagelliforme* hexane, chloroform, and butanol extract of the *in vitro* and

field-grown plant extracts. The results showed that hexane and chloroform of both *in vitro* and field-grown plant extracts possessed an IC₅₀ of more than 100 µg/mL. In contrast, the butanol *in vitro* and field-grown plant extracts failed to show cytotoxic effects on HepG2 liver cancer cells (Chan et al., 2005).

Colon cancer

The study by Setiawati et al. (2016) was done firstly to observe the cytotoxicity of *T. flagelliforme* leaves ethyl acetate extract on WiDr human colon cancer cells using MTT assay, which resulted in the IC₅₀ of 70 µg/mL, compared to celecoxib as the positive control with the IC₅₀ of 15.8 µg/mL. Next, this study observed apoptotic cell formation using a double staining method under the fluorescent microscope that showed 65% of cells undergoing apoptosis and compared with celecoxib and untreated cells, which are positive and negative control, at 88% and 1% of cell apoptosis, respectively. Lastly, the results of the COX-2 expression of this study showed a significantly higher COX-2 expression was found on untreated WiDr cells compared to the *T. flagelliforme* and celecoxib-treated cells, with celecoxib-treated cells displaying the lowest COX-2 expression ($p < 0.05$).

Squamous cell carcinoma

One study by Priyosoeryanto et al. (2020) used *T. flagelliforme* leaves ethanol extract at 120 ppm in FSCC feline squamous cell carcinoma and CSCC canine squamous cell carcinoma to observe the cell proliferation activity using the staining method. The result showed that only 35.84% and 26.84% of cell proliferation were inhibited at 120 ppm concentration for FSCC and CSCC, respectively.

DISCUSSION

The studies included in this review were mainly conducted to observe the cell proliferation activity, apoptotic activity, and cancer-related protein expression in the cancer cell. In total, there were 25 studies that observed the proliferation activity of the cells. Twenty studies used the MTT assay to observe the proliferation effect. From the MTT result, several extracts showed IC₅₀ of lower than 200 µg/mL, with 12 studies showing less than 20 µg/mL and 11 studies were less than 200 µg/mL. However, one study by Nurrochmad et al. (2011) showed a very high IC₅₀ of 632 µg/mL. According to the American National Cancer Institute (NCI) and Geran et al. (1972), the cytotoxic activity of a crude extract is considered very active if the IC₅₀ value is less than or equal to 20 µg/mL, quite active if the IC₅₀ value is 21 to 200 µg/mL, weak if the IC₅₀ value is 201 to 500 µg/mL, and inactive if the IC₅₀ value is more than 501 µg/mL. As shown in Table 1, the IC₅₀ of *T. flagelliforme* extract ranges from 2.6 to 632 µg/mL. The IC₅₀ varies due to multiple reasons such as variations in the cell lines used, the solvent used for the plant extraction, part of the plant used, treatment time, bioactive ingredients after purification, plant mutation, age of the plant, and the plant accession. Aside from the MTT study, 5 other studies used the staining method to observe the cell proliferation activity with methylene blue, trypan blue, acridine orange, and natural red staining method to observe the percentage of cell proliferation inhibition. Although both MTT and staining methods do not have a distinctive difference, the results from both methods could be presented as a percentage of either cell viability or cell proliferation inhibition. Therefore, it could be concluded that *T. flagelliforme* extract possessed quite active cytotoxicity as they possessed an IC₅₀ of fewer than 200 µg/mL, which could be beneficial to inhibit the cell proliferation in cancer cells.

Apoptosis is a programmed cell death that is important for maintaining the cell population in the tissues where it normally occurs during development and aging. Apoptosis also occurs when cells are damaged and act as a defense mechanism (Norbury & Hickson, 2001). The cell undergoes morphological changes in apoptosis, which can be observed under light and electron microscope (Häcker, 2000). Cell shrinkage and chromatin condensation can be observed in a light microscope during early apoptosis (Kerr,

Wyllie, & Currie, 1972). In electron microscopy, cell membrane blebbing can be observed, resulting in an apoptotic body (Elmore, 2007). There are two main pathways for apoptosis: extrinsic and intrinsic pathways, which are all executed by caspase-3 cleavage that results in DNA fragmentation. Cell deaths are inevitable once caspases are activated. In the intrinsic pathway, caspase-9 initiates caspase-3 cleavage, which results in apoptosis. While in the extrinsic pathway, caspase-3 was initiated by caspase-8 activation (Martinvalet, Zhu, & Lieberman, 2005). Studies by Mohan et al. (2010) and Putra et al. (2012) showed that there was an increase in caspase-3 and caspase-9 expression after cells were treated with *T. flagelliforme* extract. These findings suggest that *T. flagelliforme* extract is capable of inducing apoptosis by stimulating the enzymes that are important for initiating apoptosis. Moreover, in the cell morphological studies, cells that were treated with *T. flagelliforme* extract underwent cell shrinkage, chromatin condensation, cell membrane blebbing, and DNA fragmentation which were shown to be the signs of cell apoptosis.

Chodidjah et al. (2014) reported a decreasing expression of Bcl-2 protein in breast cancer cells after the administration of *T. flagelliforme* extract. Bcl-2 is an anti-apoptosis protein from the Bcl-2 family protein. The Bcl-2 family protein act as a primary regulator of the intrinsic apoptotic pathway (Elmore, 2007). Bcl-2 family protein is divided into pro-and anti-apoptotic proteins. The anti-apoptotic protein, such as Bcl-2 protein, as the name implies, works to inhibit the apoptosis activity in the cell, thus making cancer cells survive. The study by Chodidjah et al. (2014) confirmed that *T. flagelliforme* is capable of evading the inhibition of cell apoptosis in cancer by decreasing the expression of Bcl-2 proteins.

In the study by Putra et al. (2012), the p21 expression in breast cancer cells after treatment with *T. flagelliforme* was increased. The p21 protein controls the cell proliferation activity by inhibiting the cyclin-dependent kinase (CDK) protein complex from halting the cell cycle (Harper, Adami, Wei, Keyomarsi, & Elledge, 1993). The cell cycle was halted in order to allow the DNA repair to work on the damaged DNA (Eldeiry et al., 1993). The loss of the DNA damage checkpoint cooperates with the deficiency of Cdkn1a (Cyclin-Dependent Kinase Inhibitor 1A), which could promote the presence of abnormality in chromosome number and leads to tumor development (Shen et al., 2005). The study implied that *T. flagelliforme* could inhibit cancer development by increasing the p21 expression to undergo DNA reparation.

One example of growth factor receptors that play a role in cell proliferation is the human epidermal growth factor receptor-2 or HER2/neu. The uncontrolled activation of this receptor can result in excessive cell growth and tumorigenesis (Ménard, Pupa, Campiglio, & Tagliabue, 2003). As the HER2/neu expression decreases, no cell proliferation could occur. The study by Chodidjah et al. (2014) suggests that the cancer cells treated with *T. flagelliforme* would proliferate less as the HER2/neu expression was decreased.

Another parameter that was observed is the expression of telomerase. Telomerase is an enzyme that works to maintain the protective end of chromosomes called the telomere. In the normal human somatic cells, the telomeres shorten at each cell division, and the telomerase expression is inhibited. While in the human cancer cells, the telomere is not shortening, and telomerase is often expressed. Thus telomerase existence is essential in cancer cell proliferation (Ouellette, Wright, & Shay, 2011). The study by Purwaningsih et al. (2016) implied that both HeLa and T47D cancer cell proliferation could be evaded after treatment with *T. flagelliforme* extract as it showed a decreasing telomerase expression.

A cancer study found that COX-2 expression was abundant in colon cancer, making it an enzyme of interest for cancer treatment (Sobolewski, Cerella, Dicato, Ghibelli, & Diederich, 2010). The study conducted by Setiawati et al. (2016) observed the COX-2 expression in WiDr human colon cancer cells. COX-2 produces prostaglandin E₂ to promote cancer cell proliferation and angiogenesis. Thus, drugs with COX-2 inhibition properties succeed in preventing colon cancer growth (Sobolewski et al., 2010). The study suggests that *T. flagelliforme* treatment could prevent cancer cell proliferation by decreasing the COX-2 expression in the WiDr cells.

Cancer cell invasion was only studied in one study by Zakiyana et al. (2010) using an SP-C1 human oral cancer cell line. It is one of the hallmarks of cancer. Cancer cells could invade nearby healthy cells and

other healthy organs (Hanahan & Weinberg, 2011). The invasion is done by detaching itself from a corrupted extracellular matrix to blood circulation and lymph nodes with penetration through the basal membrane (Kai, Drain, & Weaver, 2019). In the study, *T. flagelliforme* showed a significant effect in preventing the invasion of cancer cells as the number of invasion cells decreased as the treatment concentration increased.

Tyrosine kinase plays a role in cell proliferation as a signal transducer (Paul & Mukhopadhyay, 2012). As the tyrosine kinase level is reduced, shown from the study by Chodidjah et al. (2013), the transduced signals to cell proliferation could be decreased, which can then inhibit cell proliferation. The following parameter observed is in vitro study that observed the Ki67 protein. Ki67 protein appeared (Mohan, Abdul, Abdelwahab, Al-Zubairi, Sukari, et al., 2010; Mohan et al., 2008) during the active cell cycle phases, absent during the resting cycle, and decreased drastically during the later phases of mitosis (Modlin, Moss, Chung, Jensen, & Snyderwine, 2008). This is why the Ki67 protein is used as a tumor marker and is often associated with cell proliferative activity in cancer (Brown & Gatter, 2002). The minimal reduction of tyrosine kinase and the Ki67 expression at high dose treatment in the study (400 and 800 mg/kg BW) elicits a maximal reduction effect at 200 mg/kg BW. Therefore, a saturation of active compounds from the extract on the tumor cells under the experiment condition might cause high doses of extract to be unable to inhibit the cell proliferation better (Chodidjah et al., 2013).

A study on the breast cancer volume in mice by Chodidjah et al. (2014) showed that treatment with *T. flagelliforme* extract increases the volume instead of decreasing it, contrary to the *in vitro* study result. According to this study, this occurrence was thought to be due to the mutagenic properties of flavonoids found in *T. flagelliforme*.

There are a lot of extracts used to observe cell proliferation activity in these studies; among all extracts used, it seems that dichloromethane extract showed more potent activity. Furthermore, it indicates that semi-polar compounds such as flavonoids and alkaloids (Puspitasari, Swastini, & Arisanti, 2013) that is extracted by dichloromethane might be the compounds that were the most effective in inhibiting cell proliferation where the dichloromethane extract possessed low IC₅₀ in cell lines such as WEHI-3 murine leukemia cell line (Mohan, Abdul, Abdelwahab, Al-Zubairi, Aspollah Sukari, et al., 2010), CEM-ss human T4-lymphoblastoid cell line (Mohan, Abdul, Abdelwahab, Al-Zubairi, Sukari, et al., 2010; Mohan et al., 2008), MCF-7 human breast cancer cell line (Putra et al., 2012), NCI-H23 human lung cancer cell line (Lai et al., 2008, 2010), and HS578T human breast cancer cell line (Lai et al., 2010). Conversely, ethanol extract, a universal solvent, can extract polar to semi-polar and non-polar compounds (Nurrochmad et al., 2011).

To date, there is no study regarding the anticancer activity of a specific compound isolated from *T. flagelliforme*. Hence, the compound responsible for *T. flagelliforme* anticancer activity is still unknown. Nonetheless, three bioactive compounds are identified from the ethanol extract: the phytol compound, the flavonoid compounds, and octadecanoic acid, which can also be extracted using a semi-polar solvent (Widyawati, Budianta, Kusuma, & Wijaya, 2014). A study conducted by Harhari et al. (2011) showed that the phytol compound could eliminate the cancer cell via the apoptosis pathway and from its non-specific antiproliferation trait. The flavonoid compounds from the ethanol extract studied by Priosoeryanto et al. (2020) could inhibit cell proliferation via the intrinsic apoptosis pathway. In the apoptosis process, the permeability of the mitochondrial membrane was increased to inhibit the anti-apoptotic protein expression and produce the cytochrome C to activate caspase that could trigger apoptosis (Fauzi, Norazmi, & Yaacob, 2011). Lastly, the octadecanoic acid studied by Sianipar et al. (2018) could induce apoptotic activity in breast cancer; however, with the implications of increased cardiolipin replacement and mitochondrial phospholipids reduction (Hardy et al., 2003).

CONCLUSION

In conclusion, evidence suggests that *T. flagelliforme* has potential anticancer properties and has cytotoxic activity. Most of the extracts used in the studies showed that they could reduce cell proliferation with IC_{50} of less than 200 $\mu\text{g}/\text{mL}$. Also, the semi-polar dichloromethane extract showed more potency than the polar extract and non-polar extract studied. However, there was limited data on the anticancer activity of *T. flagelliforme* semi-polar extract using a solvent other than dichloromethane. Nonetheless, ethyl acetate and chloroform extract seemingly show more potency than polar and non-polar extract. However, the difference in potency between different solvents used in semi-polar extracts (i.e., dichloromethane, ethyl acetate, and chloroform) could not be discussed and concluded due to the lack of direct comparison.

Cytotoxic activity of *T. flagelliforme* could be attributed to its effect on the upregulation of caspase-3 and -9, which are the proteins that are responsible for the initiative of apoptosis. In addition, it is also capable of decreasing the anti-apoptotic protein expression that is beneficial for the cancer cell, Bcl-2 protein. The expression of p21 protein was increased after treatment with *T. flagelliforme*, which is essential for DNA repair. In addition, the tyrosine kinase, Ki67, HER2/neu, telomerase, and COX-2 expression was decreased after treatment with *T. flagelliforme* to inhibit cancer cell proliferation. Lastly, *T. flagelliforme* can also reduce the possibility of cancer cell invasion.

Despite numerous studies conducted to evaluate the anticancer properties of *T. flagelliforme*, most of the studies were in vitro experiments. To further discover the anticancer mechanism of *T. flagelliforme* and develop it as an herbal medicine for cancer patients, a more extensive study on the anticancer mechanisms, as well as *in vivo* and clinical studies need to be conducted for further studies regarding this subject.

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