Literature Review

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The effectiveness of Fibraurea tinctoria as anticancer properties: A literature review

Efektivitas Fibraurea tinctoria sebagai komponen Anti-kanker: Literature review

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Abstract

Background: Cancer remains a leading cause of mortality globally, necessitating exploration of natural compounds for therapeutic interventions. *Fibraurea tinctoria Lour* (FT), an indigenous herb in Asia, has shown potential anticancer properties, but its efficacy and mechanisms require further elucidation.
Objectives: This review aims to summarize the existing literature on FT's anticancer effects of FT, focusing on its bioactive components, mechanisms of action, and the quantitative impact of FT extracts on various cancer cell lines.
Methods: A systematic literature search was conducted using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria across databases including Google Scholar, PubMed, and ScienceDirect, yielding six eligible studies from an initial pool of 321. These studies assessed both in vitro and in vivo effects, differing in the extract type, dosage, and treatment duration.
Results: FT extracts exhibited notable antitumor effects, with palmatine hydrochloride (PaH) demonstrating significant inhibitory activity against cancer cell proliferation. Quantitative data indicated that FT extracts can reduce cancer cell viability by 28-82.79%, with PaH-PDT emerging as a promising therapeutic strategy, effectively inducing apoptosis in multiple cancer cell lines.
Conclusion: FT extracts possess substantial anticancer potential primarily through mechanisms involving DNA damage and apoptosis induction.

Keywords:
Cancer, Fibraurea Tinctoria, Palmatine Hydrochloride, Anticancer Agents

Abstrak

Latar Belakang: Kanker masih menjadi penyebab utama kematian di seluruh dunia, sehingga perlu dilakukan eksplorasi senyawa alami untuk intervensi terapeutik. Fibraurea tinctoria Lour (FT), herbal lokal di Asia, telah menunjukkan sifat antikanker yang potensial, tetapi kemanjuran dan mekanismenya memerlukan penjelasan lebih lanjut.
Tujuan: Penelitian ini bertujuan untuk mensintesis literatur yang ada tentang efek antikanker FT, dengan fokus pada komponen bioaktif, mekanisme kerja, dan dampak kuantitatif dari ekstrak FT pada berbagai lini sel kanker.
Metode: Pencarian literatur sistematis dilakukan dengan menggunakan kriteria *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) di seluruh basis data termasuk Google Scholar, PubMed, dan ScienceDirect, menghasilkan enam studi yang memenuhi syarat dari kumpulan awal 321 studi. Studi-studi ini menilai efek *in vitro* dan *in vivo*, dengan jenis ekstrak, dosis, dan durasi pengobatan yang berbeda.
Hasil: Ekstrak FT menunjukkan efek antitumor yang menonjol, dengan palmatine hydrochloride (PaH) yang menunjukkan aktivitas penghambatan yang signifikan terhadap proliferasi sel kanker. Data kuantitatif menunjukkan bahwa ekstrak FT dapat mengurangi viabilitas sel kanker sebesar 28-82,79%, dengan PaH-PDT muncul sebagai strategi terapeutik yang menjanjikan, yang secara efektif menginduksi apoptosis pada berbagai lini sel kanker.
Kesimpulan: Ekstrak FT memiliki potensi antikanker yang substansial, terutama melalui mekanisme yang melibatkan kerusakan DNA dan induksi apoptosis.

Kata Kunci:
Kanker*, Fibraurea Tinctoria,* Palmatine Hydrochloride, Agen Antikanker

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Introduction

Cancer is the leading cause of death worldwide, with almost one in six deaths (16,8%) and one in four deaths (22,8%) from noncommunicable diseases (NCDs) around the world are attributed to cancer, making it a significant issue in terms of society, public health, and the economy in the 21st century (Bray et al., 2024). The worldwide increase in cancer prevalence can be attributed to various factors, including expanding population, aging demographics, and rapid socioeconomic development. The likelihood of developing cancer increases with age owing to the buildup of DNA damage and multistage carcinogenesis (Cho, 2017).

As cancer has become a worldwide issue, developments have been made with natural resources playing a pivotal role in cancer treatment as they modify multiple processes and decrease the likelihood of developing cancer (Muhammad et al., 2022). The principal methods used for cancer treatment include surgery, radiotherapy, and chemotherapy. However, these conventional therapies have several limitations. For instance, they eliminate both healthy and malignant cells as they target cells undergoing active division. They also increase the likelihood of tumor recurrence and disease progression. Hence, the pursuit of novel cancer therapies continues to be a rapidly expanding field of study (Aumeeruddy & Mahomoodally, 2019).

Decades of research have been conducted on scientific discoveries to cure widespread diseases, including cancer. Nature provides a plethora of resources that sustain life, including human needs for sickness treatment. For several centuries, humans have survived and developed the utilization of medicinal plants to assist longevity and recovery from illness and are now used in modern medicine (Ajlan, 2016). *Fibraurea tinctoria Lour* (FT), also known as 'Akar kuning,' is an herbal remedy that has been utilized by indigenous cultures of Asian countries. Plant roots and stems have been found to possess curative properties against several illnesses, including conjunctivitis, diarrhea, diabetes, and cancer (Utami et al., 2017).

FT has a distinct chemically active composition, known as protoberberine alkaloids and furanoditerpenoids. For protoberberine alkaloids, berberine and palmatine are its principal bioactive components (Purwaningsih et al., 2023). These chemicals function synergistically and offer a distinct therapeutic profile. FT's furanoditerpenoids comprises certain chemicals such as epi-8-hydroxycolumbin and fibaruretin B, C, D, E, and F 2023). These chemicals also facilitate anti-inflammatory activity. FT is high in concentration of secondary metabolites, which include alkaloids, flavonoids, and saponins, provides more evidence that it has the potential to inhibit the growth of cancerous cells (Ghareghomi et al., 2021; Majnooni et al., 2023; Zalizar et al., 2019). These molecules are found throughout the plant organs, and idioblasts are the main secretory structures. The presence of these metabolites in specialized structures, such as idioblasts and laticifers, indicates that a complicated mode of action may include several channels for anticancer activity (Supomo et al., 2020; Widuri et al., 2024).

The synergistic effects of the combination of berberine and palmatine produce distinctive synergistic effects. This collaboration amplifies the comprehensive therapeutic efficacy by simultaneously targeting numerous routes, mitigating the probability of pharmacological resistance and enhancing therapeutic effectiveness (Purwaningsih et al., 2023). A multi-target strategy to eliminate cancer cells involves molecular pathways related to the modulation of multiple pathways and enzyme inhibition. The modulation of multiple pathways influences the PI3K/AKT pathway, which is essential for cancer cell survival (Purwaningsih et al., 2023). Simultaneously, many signaling cascades are involved. Thus, the enzyme inhibition utilizes berberine and palmatine to engage with several protein targets and demonstrates potential in blocking enzymes essential for cancer advancement (Purwaningsih et al., 2024).

The mechanism of action of the inhibitory effects of FT has a unique chemical makeup, including furanoditerpenoids and ecdysteroid glucosides. One of the recognized compounds known as inhibitors of cytochrome P450 3A4 (CYP3A4), an enzyme involved in drug metabolism, includes palmatine and jatrorrhizine. With IC50 values of 0.9 and 2.1 μM respectively, these molecules show notable inhibitory effects that may help to explain their anticancer actions via influencing drug metabolism paths (Su et al., 2007).

Additionally, FT demonstrates antioxidant activity, which is essential in the fight against oxidative stress, a factor that has been associated with the advancement of cancer. The modest DPPH (2,2-diphenyl-1- picrylhidrazyl) scavenging action of the plant stems is attributed in great part to their high phenolic and flavonoid content in the ethanol extract. This antioxidant capacity could aid in lowering the oxidative damage to cells, thereby contributing to its anticancer action (Fathiah et al., 2024). Cytotoxic effects on cancer cell lines have been shown using DLD1 colon cancer cell lines, and in vitro experiments have shown FT's cytotoxic properties of FT. At 1600 ppm, the plant HB subfraction showed a considerable inhibitory effect, thereby lowering cell viability by 28%. This implies that some fractions of the plant could be especially successful in focusing on cancer cells (Sulistiarini, Soemardji, Elfahmi, Waluyo, et al., 2020).

Ultimately, the anticancer effects of FT are ascribed to its rich concentration of secondary metabolites and unusual chemical components, comprising cytochrome P450 inhibitors. The plant's antioxidant action and cytotoxic effects on cancer cell lines highlight its potential as a natural anticancer agent. These results emphasize the need for further investigation to clarify the processes by which FT exerts anticancer properties. Researchers believe that FT and its constituent berberine could potentially be effective in addressing oxidative stress and diabetes; however, their efficacy in cancer treatment remains unconfirmed. There is an alkaloid in FT called palmatine hydrochloride (PaH), which has been shown to effectively trigger apoptosis in colon adenocarcinoma HT-29 cells and breast cancer MCF-7 cells; thus, further studies are required (Manosroi, Akazawa, Akihisa, et al., 2015; Manosroi, Akazawa, Pattamapun, et al., 2015; Sulistiarini et al., 2020; Wu, Xiao, Zhang, Xue, Leung, Zhang, Tang, et al., 2016; Wu, Xiao, Zhang, Xue, Leung, Zhang, Xu, et al., 2016a).

Current FT studies primarily focus on OSCC, colon adenocarcinoma, and breast cancer, as cancer cells as other common cancer types remain unexplored. There is limited understanding of effectiveness across different cancer stages, lack of studies on metastatic cancers, and scarce mechanistic understanding in incomplete areas, including ROS-independent pathways, as it has not been fully explored. Limited understanding of FT resistance mechanisms, incomplete characterization of molecular targets, and interactions with other cellular pathways are unclear. There are also clinical translation challenges, under-researched aspects such as unestablished optimal dosing protocols, limited pharmacokinetic studies, lack of human clinical trials, and safety profiles in diverse patient populations.

There is a need for treatment optimization research using combination therapy, drug delivery systems, bioavailability enhancement, and treatment resistance mechanisms. Long-term follow-up studies, standardized protocols, comparative studies with conventional treatments, comprehensive studies, and complete pathway analysis including resistance mechanism investigation, interaction with the immune system, effects on tumor microenvironment, clinical development in phase I-III clinical trials, safety and efficacy in humans, and optimal treatment protocols are required. Currently, researchers need to validate and optimize FT supplements for their biological properties, and further studies, especially on their long-term effects, are required to understand their bioactivity. Although determining the effects of FT is crucial, there is currently no worldwide analytical summary of the field in published literature. By providing in-depth research, this review seeks to elaborate on the existing limitations of FT's potential of FT as an anticancer agent.

Method

To conduct this study, a comprehensive literature search was conducted using the Google Scholar, PubMed, and Science Direct databases. Publications released from 2015 to February 2024 were selected when the journal was created. The year of publication has been extended owing to the scarcity of research on the effects of FT on cancer. Research before 2015 was excluded, and research more than 2015 was included. We conducted a thorough search using systematic methods to identify the relevant studies. The search approaches were comprehensive; however, the volume of articles was restricted because of the scarcity of publications on this topic.

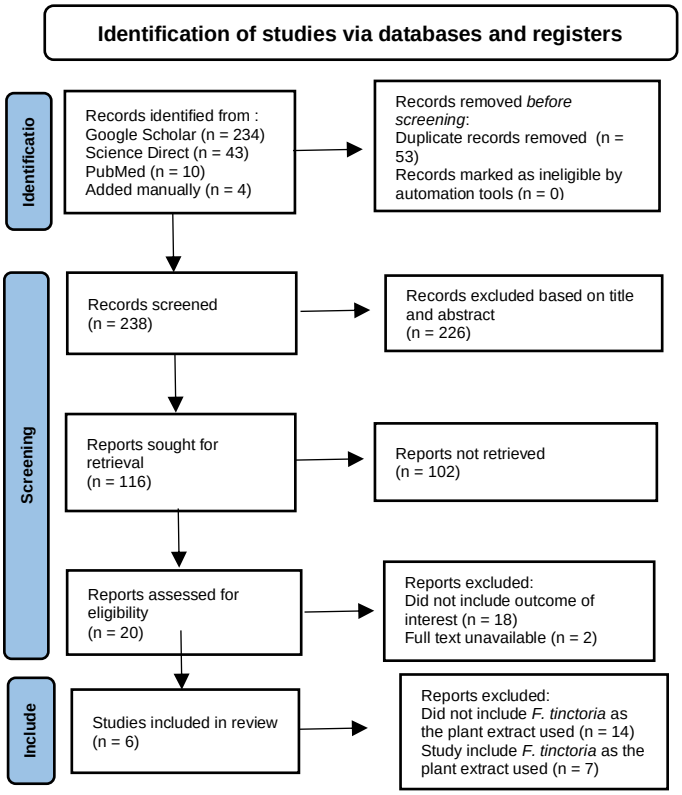

Figure 1. PRISMA flow diagram of study selection

This study adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). After removing duplicates, 238 studies were identified. The papers were analyzed and their applicability was carefully vetted. Ultimately, seven publications from the first search and four additional papers cited in the original studies were chosen for the analysis. Each study produced interesting results and conclusions. Seven studies were selected for the review.

The search query used was ((*Fibraurea tinctoria* [Title/Abstract] AND (in vitro) OR (in vivo) [Title/Abstract]) OR (in vivo [Title/Abstract] AND cancer OR anticancer) OR ((*F. tinctoria* [Title/Abstract] AND (cancer)). The name "*Fibraurea tinctoria*" was associated with the term "cancer" in the search bar. The papers reviewed were thereafter chosen based on their inclusion of all the specified information, such as the use of FT extract, a study comprising two categories of studies that focused on cancer cells: in vitro and in vivo investigations (Figure 1). No limitations were imposed on the types of cancer examined. Preclinical trials on FT's therapeutic value of FT were also included. Letters, case reports, editorials, clinical trial reviews, meeting abstracts, posters, protocols, books, and meta-analyses were excluded. The research papers were evaluated based on study population, types of FT interventions, dosages, and sample sizes. Papers published in languages other than English, or whose results had no bearing on the topic of the review, were excluded. Only full-text papers and papers to which the writer has access are included.

Table 1. The population, intervention, comparison, and outcomes (PICO) framework

Element	Details
Population	Cancer cell line Cancer cells in animal samples
Intervention	Components extracted from *Fibraurea tinctoria*
Comparison	Cell line, or cancer cells in animal with a control specimen, the utilized cell lines, the chosen concentrations of *Fibraurea tinctoria*
Outcomes	Effects of the anticancer properties, namely apoptosis rate and tumor inhibition rate

Data extraction was performed using tables to provide a summary of essential information concerning several aspects. These aspects included the author, year of publication, research methods, study sample, intervention, type of control, and effects of anticancer properties. Subsequently, a qualitative investigation focused on the effects of anticancer properties on cancer cells, specifically apoptosis and tumor inhibition rates. The scarcity of recent research on FT's anticancer effects of FT appears to be multifactorial rather than due to any single cause. Evidence from compiled journals suggests that the compound shows moderate antiproliferative activity and promising anticancer potential, particularly when using the photodynamic method (Manosroi et al., 2015a; Qi et al., 2019a; Wu et al., 2016a).

However, the limited research can be attributed to the recent discovery of its anticancer properties, regional concentration of research efforts, technical challenges in study design and implementation, competition from more established natural compounds, limited

international collaboration and funding, and lack of registered trials in major clinical trial databases. Most research has been limited to in vitro studies, lack of progression from preliminary studies to clinical investigations, and more promising medicinal plants such as Curcuma longa and Andrographis paniculata have received more research focus owing to their wider geographical distribution, better established therapeutic potential, more extensive traditional use documentation, and greater awareness in the scientific community (Manosroi, Akazawa, Pattamapun, et al., 2015a). Current evidence does not suggest that FT has "small effects" on cancer; rather, it indicates that the research field is in its early stages and requires more comprehensive investigation to fully understand its therapeutic potential.

The reviewers evaluated the risk of bias (RoB) of the papers included in the inclusion. The RoB tool developed by SYRCLE was used to evaluate the potential for bias in in vivo research investigations, and a modified version of the SYRCLE tool was used to address in vitro research (see Table 5 in the Supplementary Materials). The author previously considered the use of the Cochrane risk-of-bias tool; however, it proposed randomizing the allocation sequence. This is a barrier to in vitro evaluations, as randomization is difficult to implement with in vitro materials. According to a systematic review by Tran et al. (2021), the reviewed in vitro studies using Cochrane Collaboration, Timmer's Analysis Tool, and Oral Health Assessment Tool (OHAT) did not have a clear and detailed statement on how they reached their conclusions regarding the randomization of the trials that were included. The modified SYRCLE RoB tool was chosen because of its structured framework that can be modified for in vitro studies, its origin in preclinical animal studies, its compatibility with laboratory research rather than clinical trial tools, and its use in the assessment of key experimental parameters relevant to cell culture studies.

The modified SYRCLE RoB tool was based on a previous systematic review of in vitro studies. This was done because both in vitro and in vivo studies were included. The information on bias obtained from each study was arranged in a table, and the judgments that corresponded to it were as follows: "Yes" implies a low risk of bias, "No" indicates a high risk of bias, and "Unclear" suggests that not enough information was reported.

Table 2. Risk of bias for in vitro studies (Modified SYRCLE's RoB tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Study 1 (Sulistiarini et al., 2020)	No	Unclear	Yes	Yes	Yes	No	No	Yes	Yes	Unclear
Study 2 (Qi et al., 2019a)	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	No
Study 3 (Manosroi et al., 2015a)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Study 4 (Wu et al., 2016a)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Unclear
Study 5 (Wu et al., 2016a)	Unclear	Yes	Yes	Yes	Unclear	Unclear	No	Yes	Yes	Unclear
Study 6 (Manosroi et al., 2015a)	No	Yes	Yes	Unclear	Yes	Unclear	No	Yes	Yes	Unclear

Table 3. Risk of bias in in vivo studies (SYRCLE RoB tool).

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Study 2 (Qi et al., 2019a)	No	Yes	No	No	No	Yes	No	Yes	No	Unclear

Result

Table 4. Summary of the studies

Study	Cell line	Type of Study	Type of Extract	Dose	Duration	Findings and Conclusions
(Sulistiarini et al., 2020)	DLD1 colon cancer cell lines	In vitro study	Methanol extract, n-Hexane fraction,Ethyl acetate fraction,HA isolate, HB isolate	Concentrations of FT : 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, and 1600 ppm	Incubated for 48 hours.	Considering the IC50 values are significantly higher than the established cutoff for possible anticancer activity, the researchers conclude that the FT plant materials cell line. They do, however, imply that the plant might have a broad therapeutic range and be beneficial for further medical uses.
(Qi et al., 2019a)	human oral squamous cell carcinoma (OSCC)	In vitro and in vivo study, Photodynamic Therapy (PDT) Setup	Palmatine hydrochloride (PaH)	Dose of PaH varied with concentrations up to 16mM showing effects on cell viability : 1.2, 1.8 and 2.4 J/cm^2 (in vitro), 90 J/cm^2 (in vivo) dosage of light.	10 minutes (in vivo) and 120 seconds (in vitro)	In mice, PaH-PDT significantly slowed the growth of tumors and increased survival times. In OSCC, PaH-PDT increased p53 expression and increased ROS generation. PaH has great potential because there have been no reported detrimental outcomes or loss of weight.
(Manosroi et al., 2015a)	HT-29 cell line and cell selectivity on human skin fibroblast (HSF)	in vitro study	methanolic extracts (MEs), hexane fraction (HF), methanol-water fraction (MF), n-butanol fraction (BF), water fraction (WF)	Exact dose of FT is not explicitly stated in the provided contexts but were likely within the range tested for anti-proliferative effects against the HT-29 cancer cell line. Anti-proliferative activities of HT-29 IC 50 (µg/ml) : ME (17.13 µg/ml),	The treatment duration in the study involved incubating the cells with the samples for 24 hours. The doubling time of the human colon cancer cell line (HT-29) used in the study was	The study's conclusions demonstrated that, in comparison to its MEs, the methanol-water fraction (MF) of FT exhibited encouraging anti-proliferative properties, suggesting its potential as a source of bioactive chemicals for treating cancer.

Study	Cell line	Type of Study	Type of Extract	Dose	Duration	Findings and Conclusions
				HF >200 µg/ml, MF (9.39 µg/ml), BF (>200µg/ml) WF (>200 µg/ml) Anti-proliferative activities of HSF IC 50 (µg/ml) : ME (>100 µg/ml), HF (Not tested), MF (>100 µg/ml), BF (Not tested) WF (Not tested)	about 19 hours, so the 24-hour incubation time was chosen.	
(Wu et al., 2016a)	colon adenocarcinoma HT-29 cells and NIH-3T3 normal cells	in vitro study	Palmatine hydrochloride (PaH) (HPLC 98, CAS: 10605-02-4)	Concentrations of PaH (0, 0.04, 0.2, 1, 5, 25 µM) and irradiated with 470 nm LED light at energy densities of 3.6 and 10.8 J/cm2. PaH (1 µM) for live-cell microscopy measurements.	Duration of the test involving the evaluation of palmatine hydrochloride (PaH) on colon adenocarcinoma HT-29 cells included several time intervals for different assessments	On HT-29 cells, PaH-mediated photodynamic treatment showed notable photocytotoxicity in a way that was both light dose-dependent and dependent on PaH concentration. Following PaH-photodynamic therapy, there was a notable increase in the early and late apoptotic rates of HT-29 cells.
(Wu et al., 2016a)	breast cancer MCF-7 cells	in vitro study	palmatine hydrochloride (PaH)	Concentrations of PaH ranging from 0.04 M to 25 M. IC50 of PaH was 0.087 M at an energy density of 10.8 Jcm^2	40-minutes incubation period for cellular uptake, followed by analysis of photocytotoxicity and apoptosis induction at 18 hours post-treatment.	PaH-PDT raised the occurrence of initial and final apoptosis/necrosis and intracellular ROS levels in MCF-7 cells, and dramatically decreased the ability to survive of MCF-7 cells in a drug and light-dose dependent manner.
(Manosroi et al., 2015a)	human cervical adenocarcino	in vitro study	hexane-soluble fraction	Exact dose of FT is not explicitly stated in the provided contexts but were	24 hours for the anti-proliferative assay	In the investigation, FT exhibited anti-proliferative efficacy against human

Study	Cell line	Type of Study	Type of Extract	Dose	Duration	Findings and Conclusions
	ma (HeLa) and mouth epider mal carcino ma (KB		(HF), methanol–water-soluble fraction (MF), n-butanol-soluble fraction (BF), and water-soluble fraction (WF)	likely within the range tested for anti-proliferative effects. Anti-proliferative activity HeLa GI 50 (µg/ml) : FT were 39.61, HF were 16.9, MF were 29.4, BF were 23.3, WF were 27.9 Anti-proliferative activity KB GI 50 (µg/ml) : FT were 31.50, HF were 27.7, MF were 24.3, BF were 51.9, WF were 11.0		cervical (HeLa) and oral (KB) cancer cell lines. The findings indicate that FT's methanolic extract's anti-proliferative impacts on HeLa and KB cancer cell lines were enhanced by fractionation into various soluble fractions, underscoring the plant's prospects for additional development as a therapeutic agent against oral and cervical cancers.

Table 2 summarizes the seven publications considered in this systematic evaluation. There are six studies that are conducted in a laboratory setting (in vitro studies) (Manosroi et al., 2015a; Sulistiarini et al., 2020; Wu et al., 2016a), and one study that is conducted both in a laboratory setting and in a living organism (in vitro and in vivo study) (Qi et al., 2019a). The impact of FT on cancer activity was assessed by examining the effects of FT extracts on cancer cells in seven studies using different FT components, including palmatine hydrochloride, methanolic extracts (MEs), hexane fraction (HF), methanol-water fraction (MF), n-butanol fraction (BF), water fraction, petroleum ether extracts (FTP), chloroform extracts (FTC), and methanol extracts (FTM). RCT studies were not included in this study because of a lack of justification for RCT studies.

Given the heterogeneous nature of these studies, it would not be appropriate to conduct a meta-analysis or estimate the overall effects. Instead, the focus should be on qualitative synthesis and identifying patterns across studies while acknowledging their methodological differences. In study 1, conducted by Sulistiarini, Soemardji, Elfahmi, Iwo, et al., various components of FT extracts were tested in the DLD1 colon cancer cell line. The extracts were standardized at concentrations ranging from 6,25 ppm to 1600 ppm to maintain consistent testing conditions. The cell line was cultured in DEMB medium and the survival rate of the test materials was evaluated using a cytotoxicity assay. Following the delivery of the extract, the cells were placed in an incubator set at 37°C in an environment containing 5% CO2 for 48 h. The extract exhibited no cytotoxicity towards either normal or cancerous cells, specifically the DLD1 cell line, with the exception of the HB subfraction, which showed 28% inhibition at the highest dose of 1600 ppm.

This study clearly outlined the extraction of plant materials via reflux and subsequent fractionation. The methodology is overly simplistic and further details are required for its reliability. There is a lack of data concerning the impact on normal cells and the in vivo outcomes in this study. The nature of the study, exclusively reliant on in vitro assays, makes it impossible to derive conclusions regarding safety or efficacy. These findings underscore the need for further investigations on diverse cell lines, both cancerous and normal cell lines, and animal studies to assess toxicity and in vivo efficacy. In the second study by Qi et al., a distinct result was obtained. This study examined the effects of palmatine hydrochloride-

mediated photodynamic therapy on oral squamous cell carcinoma (OSCC) using the SCC-9 cell line. Various methods have been employed to measure the impact of treatment, including assessment of cell proliferation, cell apoptosis, cell cycle distribution, protein levels (CDK2, Cyclin E1, p53), intracellular reactive oxygen species (ROS) generation, and tumor growth inhibition. The assessment was conducted over a period of 14 days post-treatment to evaluate the long-term effects and efficacy of therapy.

This study emphasized the efficacy of PaH in triggering apoptosis and eliminating breast cancer. MCF-7 PaH-PDT treatment suppresses tumor growth and enhances survival in mice. This study presents significant findings regarding the efficacy of palmatine hydrochloride (PaH)-mediated photodynamic treatment (PDT) for oral squamous cell carcinoma (OSCC). This study provides compelling evidence for the anticancer effects of PaH in OSCC cell lines in vitro and in xenograft animal models. Study 3 was conducted by Manosroi et al. (2015), who examined the anti-proliferative effects of the methanolic extract (ME) and fractions of FT against the HT-29 cancer cell line using a sulforhodamine B assay. The results demonstrated that the methanol-water fraction (MF) of FT exhibited encouraging antiproliferative actions. However, the FT components were not explicitly specified.

In Study 4, Wu et al. (2016), researchers examined the effects of palmatine hydrochloride, derived from the rhizomes of FT, on colon cancer HT-29 cells and NIH-3T3 normal cells, which served as the control group. The treatment duration varied according to the time interval between the different assessments. Cellular uptake ranged from 0 to 120 min. Dark toxicity tests were conducted 24 h after incubation, and the effects of the photodynamic treatment were observed 18 h after treatment. The mechanism of action indicates that PaH-PDT causes apoptosis-mediated cell death in HT-29 cells, as confirmed by flow cytometry data using Annexin V/PI labeling. This study demonstrated that PaH accumulated in the mitochondria of HT-29 cells. This suggests that the principal mechanism involves mitochondrial impairment, which ultimately results in apoptosis. PaH can generate singlet oxygen ($^1O_2$) when activated by light, which is a crucial element in photodynamic treatment that produces reactive oxygen species (ROS) and induces cellular damage.

In Study 5, Wu et al. (2016a) extracted palmatine hydrochloride (PaH) from the rhizomes of FT and examined its effects on breast cancer MCF-7 cells. The cells were treated with different doses of PaH, which ranged between 0.04 M to 25 M. At the end of the treatment, the cells underwent photodynamic inactivation. This process primarily involves the elevation of intracellular ROS levels. The research indicates that PaH-mediated photodynamic therapy (PaH-PDT) largely triggers cell death through an apoptotic mechanism in MCF-7 cells. PaH mostly accumulated in the mitochondria and endoplasmic reticulum (ER) of MCF-7 cells. This research indicated that subcellular localization plays a role in PaH-mediated cell death, as these organelles are essential for cellular survival. Activation of PaH by blue light also elevates intracellular levels of reactive oxygen species (ROS) in MCF-7 cells, suggesting that ROS formation is a mechanism of cellular injury. This study highlighted the presence of active compounds such as berberine, which contributed to its cytotoxic effects.

In the last study, study 6 by Manosroi et al. (2015a) tested 23 plant samples, including FT extracts and fractions, on human cervical adenocarcinoma (HeLa) and mouth epidermal carcinoma (KB). The FT methanolic extract was fractionated into different soluble fractions, which improved its ability to inhibit the growth of HeLa and KB cancer cells. The antiproliferative assay was performed for 24 h. Additional research is necessary to separate and determine the bioactive substances responsible for the antioxidant and cytotoxic effects of FT. This will help us to gain a better understanding of its potential for therapeutic use. Consistent with earlier research, these results appear to suggest that there were no notable adverse effects or modifications to critical health markers such as vital signs, organ function, or blood counts.

All things considered, the findings point to the possibility that FT can reduce the malignant activity towards cancer cell lines DLD1 (Sulistiarini et al., 2020), OSCC (Qi et al., 2019a), HT-29 (Manosroi et al., 2015a), HT-29 (Wu et al., 2016a), MCF-7 (Wu et al., 2016a), HeLa, and KB (Manosroi et al., 2015a) by 28–82.79% without causing any appreciable adverse events according to self-report in any of the seven trials.

According to IC50 or GI50 values, Palmatine HCl utilized in photodynamic therapy had the lowest IC50 Wu et al. (2016a), followed by FT's MF (Manosroi et al., 2015a). Sulistiarini et al. (2020)

exhibited minimal potency, as evidenced by a GI50 of 1600 ppm. PaH alone shown no inhibitory effects on cells in the trials conducted by Wu et al. (2015) Sulistiarini et al. (2020) employed no standards, rendering the computation unfeasible. Wu et al. (2015a) reported the IC50 values with PaH-PDT as the reference, utilizing the laser as the control, and the activity was significantly reliant upon light activation.

The cell viability study demonstrated that PaH in darkness exhibited little anti-proliferative characteristics; nevertheless, when subjected to light irradiation, it significantly enhanced toxicity towards HT-29 cells. Manosroi, Akazawa, Akihisa, et al. reported that FT's ME exhibited minimal efficacy, with GI50 values demonstrating potency inferior to that of cisplatin by a factor of 0,06 0,14. The MF fraction of FT exhibited enhanced activity with a GI50 of 9,39 µg/ml, demonstrating greater potency than conventional medicines, being 1.82 times more effective than cisplatin. However, Wu et al. (2015a) did not compare the use of conventional medications.

Discussion

This systematic review explored the effects of FT on cancer cell populations using different types of extracts, concentrations, and isolation. The study design and results are mostly used in vivo to generate changes in the cancer cells. The overall results of these studies show that FT has potential as an anticancer agent. In one of the reviewed studies, the experiment relating to majority of FT substances tested did not show sufficient potency, with no cytotoxicity towards either normal or cancerous cells, specifically the DLD1 cell line at the concentration of extract, fraction, or isolate material tested to be considered potentially effective as anticancer agents based on the established criteria, except for the HB sub-fraction, which showed 28% inhibition against DLD1 colon cancer cells (Sulistiarini et al., 2020). In vivo studies using animal models are required to validate the anticancer activity of FT extract and provide new insights into its potential therapeutic applications in colon cancer treatment. For the other six studies reviewed, most decreased the activity of cancer cells in all the six studies in which mainly cell proliferation and apoptosis were measured. Specific percentage values of cancer reduction by FT were not explicitly provided in most studies.

Subjects with several types of cancer received FT therapy using various approaches, with variances in the extract types, doses, and length of treatment, as shown in Table 2. However, most reviewed studies did not provide information on the side effects of FT. There is insufficient information on the implications of prolonged supplementation, because most studies did not highlight the safety and efficacy of FT and focused on brief studies. Furthermore, only one of the evaluated studies was conducted in vivo, representing the intricate nature of live species. There are possibilities that FT is still yet to be studied, as sources for FT are limited; therefore, scientists prefer reduced risks and safer and more effective data gathering. There are also gaps in the long-term efficacy, long-term toxicity, and potential resistance development of FT, as most reviewed studies did not specify the long-term effects and mostly focused on short-term assessments. As there is no published literature on the possible negative consequences of FT, extensive information addressing its potential for harm is limited to examined trials and other published studies. As it is difficult to know whether this product affects or exacerbates specific issues, it is advisable to refrain from using large quantities of FT or utilizing it without competent health advice.

Efficacy

Sulistiarini et al. failed to demonstrate any notable in vitro anticancer efficacy against DLD1 cells by using the methodology and technology employed. The study concluded that extracts and fractions of FT, specifically, exhibited no significant in vitro cytotoxicity against DLD1 colon cancer cells, with the majority of results approximating the negative control values for survival rate percentage, excluding the HB fraction (Sulistiarini. However, this study lacks safety data. Another study by Qi et al. presented robust preclinical evidence indicating that PaH-PDT is an effective method for treating OSCC both in vitro and in vivo.

The results of the cell viability, cell migration, cell cycle, and apoptosis assays support this assertion. A study by Qi et al. indicated that PaH exhibits toxicity towards normal cells despite the fact that PaH-PDT demonstrated greater efficacy in targeting cancer cells relative to normal cells, suggesting a degree of specificity for the tested

cancer cell line. In vitro experiments demonstrated a significant reduction in the migration of cells subjected to PaH-PDT, indicating a decreased risk of metastasis. The in vitro study also indicates a notable photocytotoxic effect of palmatine hydrochloride (PaH) in conjunction with light irradiation on HT-29 colon cancer cells (Wu et al., 2016). This impact was demonstrated to be dependent on both PaH concentration and light dosage. As the concentration of PaH and light energy increased, the cytotoxic effect amplified.

The authors demonstrate that a combination of 5μM PaH and a light energy dose of 10.8 J/cm² produced the greatest degree of cell killing (Wu, Xiao, Zhang, Xue, Leung, Zhang, Xu, et al., 2016). Other studies have indicated that palmatine hydrochloride (PaH), in conjunction with light irradiation, exhibits a notable photocytotoxic effect on MCF-7 breast cancer cells in vitro (Wu et al., 2016a). This effect depends on both concentration and light dosage (Wu et al., 2016a). They determined that the IC50 of PaH in MCF-7 cells was 0.087 μM at a light-energy dose of 10.8 J/cm², suggesting that a relatively low concentration of PaH is necessary for cell inhibition in conjunction with light irradiation. The research indicates that PaH-mediated photodynamic therapy (PaH-PDT) triggers cell death through an apoptotic mechanism in MCF-7 cells (Wu et al., 2016a).

PaH mostly accumulated in the mitochondria and endoplasmic reticulum (ER) of MCF-7 cells. The research indicates that Subcellular localization plays a role in PaH-mediated cell death, because these organelles are essential for cellular survival (Wu al., 2016a). Activation of PaH by blue light also elevates intracellular levels of reactive oxygen species (ROS) in MCF-7 cells, suggesting that ROS formation is a mechanism of cellular injury. However, therapeutic approaches that enhance ROS production can selectively induce cell death in malignancies, as opposed to normal cells, as cancer cells already have elevated ROS levels (Galadari et al., 2017). To counteract the possibility of excessive ROS formation, the utilization of reactive oxygen generating anticancer agents in conjunction with antioxidant inhibitors may be advantageous in cancer therapy (Galadari et al., 2017).

Mechanism of Action

Sulistiarini et al., 2019) a mechanistic assessment of the HB fraction. PaH-PDT induces cell death by apoptosis, cell cycle arrest, reactive oxygen species production, and elevated expression of P53 (Qi et al., 2019b). (Manosroi, Akazawa, Pattamapun, et al., 2015b) evidence that this activity is linked to the induction of apoptosis (Manosroi, Akazawa, Pattamapun, et al., 2015b). Nevertheless, this effect has not been quantified or assessed using molecular markers or bioassays to enhance our understanding of the underlying mechanisms. This indicates that the therapy induced apoptosis through several mechanisms, including the generation of reactive oxygen species (ROS) and expression of p53 in the cell (Qi et al., 2019b).

Regarding mechanisms, studies by (Qi et al. (2019b), Wu et al. (2016b), and Wu et al. (2016c) are far more conclusive than those conducted by (Manosroi et al. (2015b), particularly in comparison with the study by (Sulistiarini et al. (2020), which resulted from the appropriate utilization of model systems capable of evaluating both cellular proliferation and cellular mechanisms.

FT compounds (palmatine, jatrorrhizine) inhibit CYP3A4 (IC50: 0.9 μM and 2.1 μM respectively) (Su et al., 2007). There are potential interactions with chemotherapeutic drugs that are metabolized by CYP3A4. Chemotherapy combinations are still unknown; hence, studies on interactions between cisplatin and doxorubicin, impact on drug resistance mechanisms, and effects on treatment efficacy are needed. Careful monitoring is needed when combined with conventional treatment. Currently, there is no updated research on FT combined with conventional treatment.

Pharmacokinetics and Drug Delivery

Recent studies have revealed substantial deficiencies in the comprehension of FT pharmacokinetics. The absorption, distribution, metabolism, and excretion (ADME) characteristics require thorough investigation for clinical application. The restricted bioavailability of herbal components, especially alkaloids present in FT, requires novel delivery methods. Recent investigations indicate that protoberberine alkaloid (PA) compounds, one of the principal components of FT, demonstrate low oral bioavailability owing to significant first-pass metabolism and P-glycoprotein-mediated efflux (Zhang et al., 2018). However, recent studies have developed novel formulations and new formulations of berberine,

absorption enhancers, P-gp inhibitors, structural modification of berberine, and fabrication of berberine salts and cocrystals to mitigate the bioavailability problem (Cui et al., 2024; Solnier et al., 2023).

Although current data show cellular localization in the mitochondria and endoplasmic reticulum (ER), broader distribution patterns remain unclear. A study on MCF-7 breast cancer and HT-29 colon cancer cells demonstrated PaH (palmatine hydrochloride) accumulation in mitochondria and ER, demonstrated the importance of these organelles in the compound's mechanism of action, and used fluorescence microscopy for localization (Wu et al., 2016a; Wu et al., 2016a). While mitochondria and ER are confirmed locations, it is imperative to acknowledge that other cellular locations have not been definitively excluded. Current studies have primarily focused on the mitochondria and endoplasmic reticulum. The mitochondria and endoplasmic reticulum are known for their roles in cellular energy production, protein synthesis, and apoptotic pathways (Lee & Min, 2018). Recent studies using advanced imaging techniques have revealed that plant alkaloids can exhibit specific tissue distribution patterns that significantly impact their therapeutic efficacy, such as matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), which provides spatial distribution information, preserves tissue integrity during analysis, allows for metabolite visualization without complex samples, and has been used in spatial distribution and biosynthetic pathways of dendrobine in dendrobium stems (Yuan et al., 2024).

In metabolic and drug interactions, CYP3A4 enzyme interactions represent only one aspect of the FT metabolic profile. CYP3A4 and CYP3A5 are responsible for the metabolism of approximately 30–50% of all known medicines. Hence, genetic variants may result in greater medication toxicity or reduced therapeutic effects, necessitating dosage modifications based on genetic profiles (Zhang et al., 2024). Further studies are needed to complete metabolic pathway mapping, drug-drug interaction studies, and the identification of active metabolites.

One of the known metabolic profiles of enzyme interactions is an isoquinoline alkaloid, known as berberine (a known compound in FT), that can inhibit CYP3A4 activity. Utilizing the polyubiquitination pathway, berberine is able to suppress the activity of CYP3A4 by downregulating PXR and accelerating its destruction (Feng et al., 2021). This inhibition may alter the pharmacokinetics and the potential of other drugs to increase their levels and toxicity. In terms of the safety profile, there is still a lack of toxicity analysis of FT. It is crucial to note that in vitro cytotoxicity data alone are insufficient for determining human safety, as they only measure direct cell damage, do not account for complex biological interactions, and cannot predict organ-specific toxicities. For in vivo studies, while there is mention of mouse models in antimalarial research, there are no specific safety data regarding organ damage or weight loss effects.

Methodological Consistency

A study conducted by Sulistiarini et al. (2020) exhibited minimal dependability owing to inadequate extraction methodology, restricted testing for singular concentrations, and inconclusive findings. A study by Qi et al. (2019b) demonstrated high methodological rigor and quality using flow cytometry and molecular markers to evaluate their impact. Nevertheless, it precludes a direct comparison with alternative extract types. Manosroi et al. (2015b) conducted a well-constructed study, although it employed a rudimentary method for cell evaluation and offered restricted mechanisms for action assessment. The study by Qi et al. (2019b) used a robust methodology and was accompanied by a thorough technical assessment, although a definitive comparison with other chemicals may be unattainable.

A primary constraint is the absence of an in vivo model or comprehensive assessment of safety and toxicity. Restricted cellular models constitute a significant constraint that hinders the ability to draw definitive conclusions. The most reliable results from in vivo experiments pertain to palmatine hydrochloride (PaH-PDT), which does not directly reflect the effects of plant extracts. Certain analyses in this study rely on a combination of GI50 and IC50 values, which are limited for comparative analysis.

Safety

In the first study by Sulistiarini et al. (2020), the authors asserted an unsupported claim regarding the "therapeutic range, using references from other studies. Consequently, the safety profile could not be determined based on this investigation alone. The evident absence of safety data, restricted in

vitro efficacy against DLD1 cells utilizing the chosen approach, and necessity for improved and rigorously controlled investigations to facilitate a comprehensive scientific understanding of FT and its characteristics. PaH is toxic to NIH-3T3 embryo fibroblasts in vitro, despite PaH-PDT demonstrating enhanced specificity for cancer cells compared to normal cells, indicating a degree of selectivity (Wu et al., 2016c). PaH-PDT also effectively eradicates colon cancer cells in vitro by causing mitochondrial-mediated apoptosis through mechanisms involving singlet oxygen generation upon light exposure (Wu et al., 2016c).

The results depended on the concentration and light dosage, and the treatment appeared to be specific to the cell model, exhibiting no harmful effects on the regular cell line. These studies by Wu et al. (2016c) present preliminary evidence that PaH may serve as a photosensitizer, as PaH has significant photokilling activity on colon adenocarcinoma HT-29 cells and induces cell apoptosis upon photodynamic treatment; thus, further investigation is required. The authors present compelling evidence for the in vitro lethal effects, mitochondrial localization of PaH, and the generation of singlet oxygen following cell irradiation with visible light, along with a mechanism of action that entails cell death (Wu et al., 2016c). Nonetheless, they distinctly illustrate the deficiencies that warrant examination in the subsequent phase of the study regarding the application of data in clinical practice.

There is also limited evidence that toxicity in vivo side effects are not discernible in mice following PaH-PDT in a study by Qi et al. (2019b), which showed no significant weight loss or decline in their overall behavior. A study demonstrated that PaH, in the absence of light irradiation, exhibited no substantial cytotoxicity towards HT-29 cancer cells or normal NIH-3T3 cells at doses of up to 5 µM and 25 µM, respectively, after 24 h of incubation (Wu et al., 2016c).

The xenograft mouse model was immunocompromised, resulting in limited data regarding the effects of PaH-PDT on immune responses in a study by Qi et al. (2019b), a study utilizing head and neck squamous cell carcinoma in immunocompromised mouse models showed weaknesses despite success in their reliability in tumor initiation and rapid tumor progression, as these models still possess certain disadvantages (Lei et al., 2016). The rates of invasion and metastasis are extremely unclear and variable, ranging from extremely low to significantly high because transplantation methods, hosts, and transplantation sites are all distinct, and failure to replicate the in vivo inflammation and immune surroundings (Lei et al., 2016). Thus, not every author has investigated the safety parameters in vivo. Consequently, based solely on these data, it is infeasible to ascertain safety parameters for clinical use. A comprehensive assessment of safety characteristics must incorporate animal models and human trials as the lack of such data diminishes the significance of the findings.

Conclusion

A systematic investigation of *Fibraurea tinctoria* (FT) revealed significant anticancer potential, particularly through its bioactive compound, palmatine hydrochloride (PaH), in photodynamic therapy applications. Notably, PaH-mediated photodynamic has emerged as the most promising approach, showing significant tumor growth inhibition in both in vitro and xenograft models while maintaining minimal effects on normal cells.

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