ACTA VETERINARIA INDONESIANA
P-ISSN 2337-3202, E-ISSN 2337-4373

Research

Vol. 13, No. 2: 167–174, Juli 2025

Antitumor Activity of *Typhonium flagelliforme* Ethanol Extract Nanoemulsion in DMBA-Induced Sprague Dawley Rats

Dian Cipta Rini[1], Bambang Pontjo Priosoeryanto[2*], Riki Siswandi[3], Lina Noviyanti Sutardi[4]

[1]Graduate Program of Animal Biomedical Sciences, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[2]Division of Veterinary Pathology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[3] Division of Veterinary Surgery and Radiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[4] Subdivision of Pharmacy, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
*Corresponding author: bpontjo@apps.ipb.ac.id
Received: 16 October 2024, Approved: 1 June 2025

ABSTRACT

Tumors are a leading cause of disease-related deaths in humans and companion animals, including dogs and cats. Typhonium flagelliforme, recognized in Indonesian traditional medicine, exhibits notable antitumor properties, such as inhibiting tumor cell proliferation and enhancing immune response. This study aimed to formulate T. flagelliforme into a nanoemulsion to improve its antitumor eflects and assess its efficacy in Sprague Dawley rats induced with the carcinogen 7,12-dimethylbenz (a) anthracene (DMBA). The nanoemulsion was formulated using the inversion phase method, and its particle size and stability were analyzed using SEM. Phytochemical screening identified active compounds, and toxicity was assessed using the brine shrimp lethality test. In vivo experiments involved five rat groups: untreated (NTC), nanoemulsion solvent (Solv), and three treatment groups receiving 25 µg/kg (TF25), 50 µg/kg (TF50), and 100 µg/kg (TF100) doses of the nanoemulsion, administered via intralesional injection. Results showed significant tumor size reduction in treatment groups compared to controls ($p<0.05$), with TF25 exhibiting the most eflective antiproliferative activity. Increased body weight across groups indicated low toxicity. The study concludes that T. flagelliforme nanoemulsion eflectively reduces tumor size and angiogenesis, demonstrating its potential as an antitumor agent.

Keywords: Antitumor, DMBA, nanoemulsion, rat, *Typhonium flagelliforme*

ABSTRAK

Tumor merupakan salah satu penyebab utama kematian terkait penyakit pada manusia dan hewan peliharaan, termasuk anjing dan kucing. *Typhonium flagelliforme* yang dikenal dalam pengobatan tradisional Indonesia memiliki sifat antitumor seperti menghambat proliferasi sel tumor dan meningkatkan respons imun. Penelitian ini bertujuan untuk memformulasi *T. flagelliforme* menjadi nanoemulsi untuk meningkatkan efek antitumornya dan menilai efikasinya pada tikus *Sprague Dawley* yang diinduksi dengan karsinogen 7,12-dimethylbenz-(a)anthracene (DMBA). Nanoemulsi diformulasikan menggunakan metode fase inversi dan ukuran serta stabilitas partikelnya dianalisis melalui *Scanning Electron Microscope*. Penapisan senyawa fitokimia dilakukan untuk mengidentifikasi senyawa aktif dan toksisitas dinilai menggunakan *Brine Shrimp Lethality Test*. Percobaan *in vivo* melibatkan lima kelompok tikus: kelompok tanpa perlakuan (NTC), pelarut nanoemulsi (Solv), dan tiga kelompok perlakuan yang menerima dosis 25 µg/kg (TF25), 50 µg/kg (TF50), dan 100 µg/kg (TF100) nanoemulsi, yang diberikan melalui injeksi intralesional. Hasil menunjukkan penurunan ukuran tumor yang signifikan pada kelompok perlakuan dibandingkan dengan kelompok kontrol ($p<0.05$), dengan TF25 menunjukkan aktivitas antiproliferatif paling efektif. Peningkatan berat badan pada semua kelompok menunjukkan toksisitas yang rendah. Penelitian ini menyimpulkan bahwa nanoemulsi *T. flagelliforme* secara efektif menurunkan ukuran tumor dan angiogenesis, menunjukkan potensinya sebagai agen antitumor.

Kata kunci: Antitumor, DMBA, nanoemulsi, tikus, *Typhonium flagelliforme*

INTRODUCTION

Tumors in companion animals are characterized by abnormal, uncontrolled cell proliferation due to genetic mutations affecting proliferation, apoptosis, and differentiation (Priosoeryanto 1994; Lewandowska *et al.* 2019). These tumors often arise in the skin and mammary glands of cats and dogs, with increasing incidence observed in humans (Ruple and Bonnett 2019; Rachmawati 2020). Conventional treatments, such as surgery, chemotherapy, and radiotherapy, often have significant side effects and face challenges like resistance to treatment (Mathewos *et al.* 2020; Wang *et al.* 2019). Therefore, alternative therapies, including herbal medicine, are gaining interest (Priosoeryanto *et al.* 2020).

Indonesia's rich biodiversity includes medicinal plants, such as *Typhonium flagelliforme*, known for its anticancer properties (Sianipar *et al.* 2020). Components like flavonoids and alkaloids in *T. flagelliforme* exhibit antiproliferative effects against various cancers and antiangiogenic properties (Priosoeryanto *et al.* 2020). However, the therapeutic potential of herbal bioactives is often limited by poor bioavailability and membrane permeability (Harwansh *et al.* 2019).

Nanotechnology, particularly nanoemulsions, can enhance drug delivery, improving targeting and efficacy while reducing side effects (Veselov et al. 2022; Preeti et al. 2023). This study aimed to evaluate the antitumor activity *of T. flagelliforme* ethanol extract nanoemulsion in rats with DMBA-induced skin tumors.

MATERIALS AND METHODS

Ethical Approval

The research was conducted from April 2022 to April 2023. The research has been approved by the Animal Ethics Commission of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia, with certificate no. 025/KEH/SKE/IX/2022.

Study Period and Location

The research was conducted from April 2022 to April 2023 at the Pharmacy Laboratory, Pharmacy Subdivision of the School of Veterinary Medicine and Biomedical Sciences, IPB University (SVMBS-IPB), Division of Veterinary Surgery and Radiology, SVMBS-IPB, Division of Veterinary Pathology Laboratory, SVMBS-IPB, and the Veterinary Teaching Hospital (VTH) SVMBS-IPB, Bogor-Indonesia, D'Animaux Veterinary Clinic Jakarta-Indonesia, Nanoparticle Laboratory of Standardization of Agricultural Instrumentation Agency (BSIP) Bogor-Indonesia, and the Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor-Indonesia.

Nanoemulsion Formulation

Nanoemulsions were produced using the phase inversion technique or low-energy phase homogenization according to the standard method (Ostertag et al. 2012). This technique involves forming a water-in-oil emulsion and then inverting the phase to an oil-in-water emulsion. Ethanol extract of *T. flagelliforme* (1%) was placed into a glass beaker and mixed with 1% DMSO until dissolved, then 1% Tween 80 was added. 20% glycerol was added under magnetic stirring at 200 rpm. The mixture was gradually added with 78% of distilled water while stirring to achieve the final emulsion volume of the formulation. While distilled water was being added, the speed of the stirrer was also increased to 750 rpm for 15 minutes to form the organic phase. The formed nanoemulsion was transferred to sample bottles, and its emulsion stability was analyzed up to the tenth day.

The nanoemulsion was analyzed using a particle size analyzer (PSA) to measure particle size and polydispersity index (PDI), and particle stability was further assessed using a scanning electron microscope (SEM).

Phytochemical Screening

Phytochemical screening was conducted to analyze the nanoemulsion for the presence of secondary metabolite compounds, such as flavonoids, alkaloids, tannins, steroids, triterpenoids, and saponins, according to standard methods by Harborne (1996).

Brine Shrimp Lethality Test

The brine shrimp lethality test (BSLT) was performed according to standard procedures (Meyer et al. 1982). BSLT was employed to ascertain the lethal concentration 50 (LC_{50}) of ethanol extract nanoemulsion derived from *T. flagelliforme*. The test began by preparing artificial seawater, consisting of distilled water mixed with salt to simulate marine conditions. Artemia salina Leach eggs were then hatched in a darkened container filled with seawater and equipped with aeration. After 48 hours, the hatched larvae were divided into groups exposed to varying concentrations of *T. flagelliforme* ethanol extract nanoemulsion: 10 ppm, 100 ppm, 500 ppm, and 1000 ppm. Each concentration group was replicated thrice, with 10 larvae per test tube. The tubes were illuminated, and larvae mortality was observed and recorded hourly over 24 hours. Larvae were considered dead if they exhibited erratic movements, spun in place, or remained immobile for 10 or more seconds. After 24 hours, the percentage

mortality was calculated for each concentration group. Probit analysis was then used to determine the LC_{50}, providing a quantitative measure of toxicity and establishing a foundation for further toxicological investigations.

Experimental Design

The *in vivo* evaluation of *T. flagelliforme* antitumor property was performed in Sprague-Dawley (SD) rats. Before tumor induction, 25 SD rats, aged 30 days and weighing 70–80 grams, were acclimatized for two weeks. During this period, they were also given anthelmintic pyrantel pamoate (15 mg/kg BW, orally) once, antiparasitic selamectin (25 mg/kg BW, topical) weekly, and antibiotic amoxicillin (50 mg/kg BW, orally) for three consecutive days. The rats were housed inside well-perforated cages in a temperature-controlled room in the Laboratory Animal Unit of VTH SVMBS-IPB. After the acclimatization, the rats underwent the skin tumor induction protocol for twelve weeks and followed by the treatment protocol for four weeks. They were provided with standard rat pellets and had ad libitum access to food and water. The rats were weighed weekly as a growth soundness indicator.

Tumor Induction and Treatments

Tumor induction (day 0) was performed on all groups using DMBA according to the standard method (Muti'ah *et al.* 2016). DMBA dissolved in acetone as a solvent and was administered subcutaneously on the rat's back twice a week for 12 weeks at a dose of 25 µg/0.05 mL per rat. The induction area was shaved and marked before treatment. Rats were observed until tumor masses formed. At the end of the induction phase (day 84), the induced tumor was measured in length and width, as it will be compared after the end of the study.

Rats with established tumors were randomly assigned to five treatment groups: an untreated control (NTC), a nanoemulsion solvent treatment (Solv), and three groups receiving *T. flagelliforme* nanoemulsion, marking the beginning of the treatment phase on day 85. The NTC group received no treatment. Solv group received 0.05 mL of nanoemulsion solvent by intratumoral injection. The three *T. flagelliforme* nanoemulsion groups (TF25, TF50, and TF100) received intratumoral injections of *T. flagelliforme* nanoemulsion at doses of 25 µg/kg BW, 50 µg/kg BW, and 100 µg/kg BW, respectively. All treatments were administered daily for three consecutive days each week over four weeks. During this treatment phase, each rat in the Solv, TF25, TF50, and TF100 groups received a total of 12 intratumoral injections.

Clinical Evaluation of Anti-Tumor Activity

Throughout the treatment phase, tumor diameter was measured weekly with calipers in both vertical and horizontal dimensions. Rat body weight, appetite, drinking and defecation habits, urination, and pain conditions were monitored daily. Rat body weight was measured twice a week using a digital scale. At the end of the treatment phase (days 113), the rats were anesthetized with ketamine and xylazine and then euthanized by cardiac puncture. Skin tumors were collected for histopathological examination.

Histopathological examination

Histopathology slides of skin tumors (stained with Haematoxylin and Eosin) were examined under a light microscope using a 40X and 100X objective magnification. Observations were focused on changes in skin structure, cell morphology, and the diagnosis of the formed tumor. The mitotic index was determined by counting the number of cells in mitosis and dividing it by the total number of cells in 10 fields of view (Mao *et al.* 2023). The angiogenesis index was calculated by counting the number of micro blood vessels per 1,000 cells in the tumor area, following the standard method (Prakash *et al.* 2022).

Statistical Analysis

The comparison of body weight and tumor size between groups was evaluated by ordinary one-way ANOVA, followed by Dunnett's post hoc analysis to compare each group with the NTC group. The comparison of mitotic and angiogenesis index was performed by the Kruskal-Wallis test and Dunn's multiple comparison test. The post hoc test was performed to compare each group against NTC. A nominal significance level of 5% (α=0.05) was used for all statistical tests. The results were expressed as the mean value with standard error of the mean.

RESULTS

Nanoemulsion

The particle size analysis (Z-Average) result showed a value of 145.7 nm, indicating that the particle size of the selected formula falls into the nanoparticle category. The polydispersity index (PDI) of the selected formula was 0.368.

Phytochemical Screening

The phytochemical screening showed positive results for all reagents, indicating that the nanoemulsion formulation of ethanol extract from *T. flagelliforme* contained alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins. The result is presented in Table 1.

Table 1 *Phytochemical screening results of T. flagelliforme nanoemulsion*

Test	Observation
Alkaloid (Mayer)	(+)
Alkaloid (Wagner)	(+)
Alkaloid (Dragendorf)	(+)
Flavonoid	(+)
Steroid dan terpenoid	(+)
Saponin	(+)
Tannin	(+)

Note: (+) Present

Brine Shrimp Lethality Test

BSLT exhibited an LC_{50} value of 384.41 ppm. The results indicate that the nanoemulsion of ethanol extract from *T. flagelliforme* exhibits high bioactivity.

Body Weight

There was an increase in body weight, which is characterized by changes in weight pre-treatment and post-treatment (Δ body weight) in all groups. The results in Figure 1 showed that there is no significant delta difference in body weight between treatment groups and NTC.

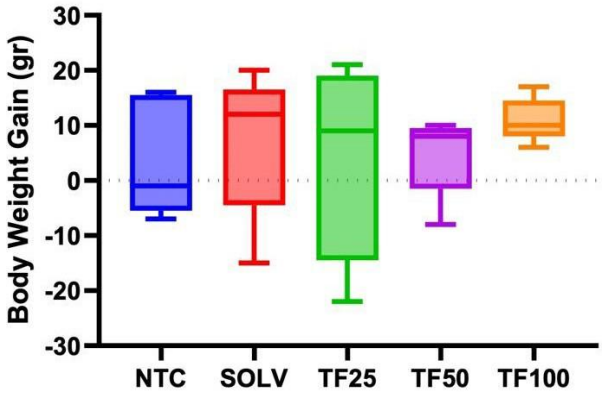

Figure 1 Rat's body weight.

Gross Lesion

The tumor lesions caused by DMBA injection in the dorsal skin of the rats were visibly apparent and palpable. The lesions formed in this study include alopecia or baldness, reddish lesions with crust, necrosis, and nodules (Figure 2).

The mean values of Δ skin tumor size are presented in Figure-3. Negative values indicate an increase in tumor size, while positive values indicate a decrease. Statistical analysis using one-way ANOVA followed by Dunnett's post hoc analysis showed that the mean Δ values for treatment groups TF25, TF50, and TF100 were significantly different compared to the control group NTC, with both TF25 and TF100 being highly significant ($p<0.001$). All groups treated with *T. flagelliforme* nanoemulsion experienced a reduction in tumor size. The largest reduction in tumor size occurred in group TF100, followed by TF25 and TF50.

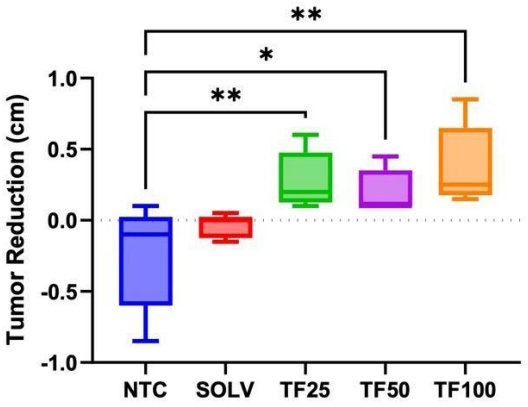

Figure 3 Tumor size reduction *= $p<0.05$; **= $p<0.01$.

Histopathology

Microscopic examination confirmed four tumor types in the lesions (Figure 4): anaplastic sarcoma, squamous cell carcinoma (SCC), myosarcoma, and fibrosarcoma. Rats with anaplastic sarcoma showed alopecia and reddish, necrotic lesions, along with epidermal hyperplasia and anaplastic cells with large, hyperchromatic nuclei. Intercellular bridges and angiogenesis were also present. SCC rats exhibited alopecia and characteristic keratin pearls, along with spindle and anaplastic cells. Myosarcoma was marked by alopecia and muscle fiber thickening, while fibrosarcoma presented as skin nodules with pleomorphic and spindle cells, along with abundant angiogenesis.

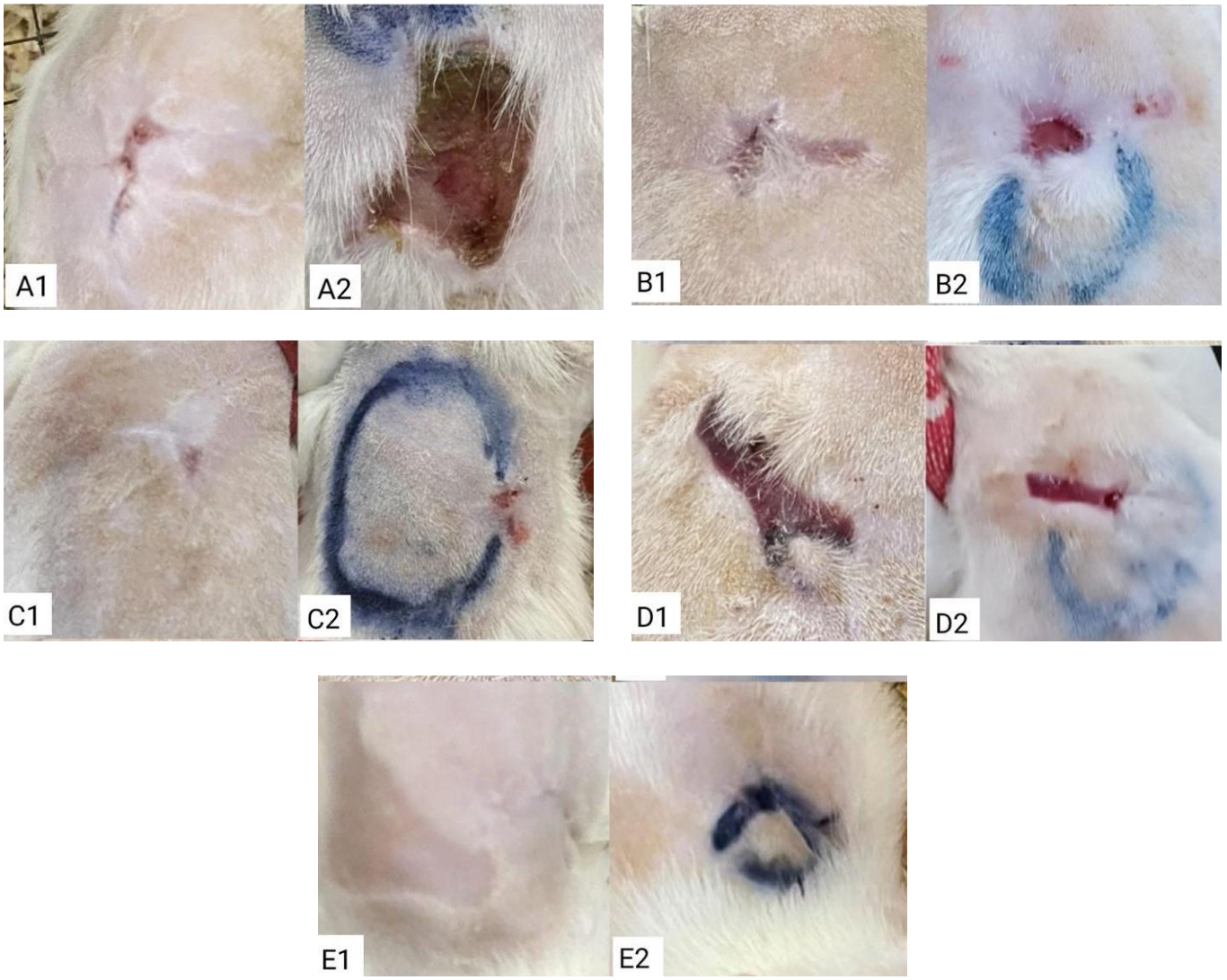

Figure 2 Gross lesion of skin tumor. A1: NTC pre-treatment: reddish lesion with scars; A2: NTC post-treatment: reddish wound lesion with nectrotic area; B1: Solv pre-treatment: reddish lesion; B2.: Solv post-treatment: nodule with open wound; C1: TF25 pre-treatment: nodule with wound; C2: TF25 post-treatment: skin with healed wound and no nodule; D1: TF50 pre-treatment: reddish lesion with open wound and necrotic area and nodule; D2: TF50 post-treatment: reddish lesion with sterile wound and no nodule; E1: TF100 pre-treatment: alopecia area with nodule; E2: TF100 post-treatment: scars with no nodule.

Statistical analysis using the Kruskal-Wallis test and Dunn's multiple comparison test revealed significant differences in the mitotic index among treatment groups TF25, TF50, and TF100 compared to NTC, with TF25 showing high significance ($p<0.001$). All groups treated with *T. flagelliforme* nanoemulsion exhibited reduced mitotic cell counts, with TF25 having the lowest index, followed by TF50 and TF100 (Figure 5). The angiogenesis index also showed significant differences for TF100 compared to NTC ($p<0.05$), while TF25 and TF50 did not differ significantly from NTC but demonstrated decreased angiogenesis (Figure 6).

Discussion

DMBA (7,12-dimethylbenz[a]anthracene) is a potent carcinogen known for inducing DNA mutations through free radical formation. It serves as a model for testing antitumor agents in vivo. Antitumor agents typically work by inhibiting cell proliferation and promoting apoptosis, essential for cellular balance, althoughthisprocesscan be disrupted byantiapoptotic proteins produced by mitochondria (Priosoeryanto *et al.* 2020). Moreover, effective antitumor compounds may neutralize reactive metabolites generated during carcinogenesis (El Yaagoubi *et al.* 2021).

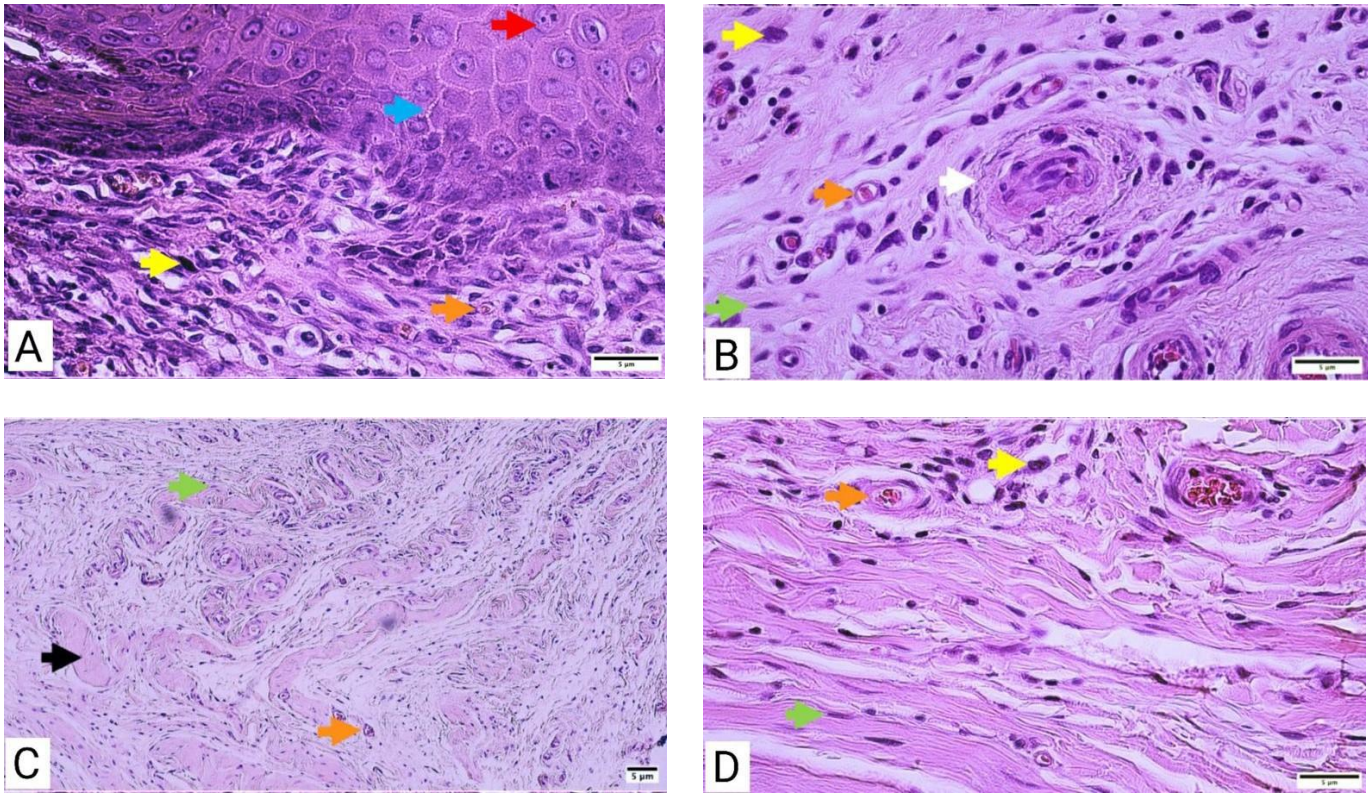

Figure 4 Gross lesion and histopathology of skin tumor: A. Anaplastic sarcoma; B. Squamous cell carcinoma (SCC); C. Myosarcoma; D. Fibrosarcoma. The red arrow indicates a mitotic cell; the blue arrow indicates an intercellular bridge; the yellow arrows indicate anaplastic cells; the green arrows indicate spindle cells; the orange arrows indicate blood vessels; the white arrow indicates a keratin pearl; the black arrows indicate hyperplastic muscular cells.

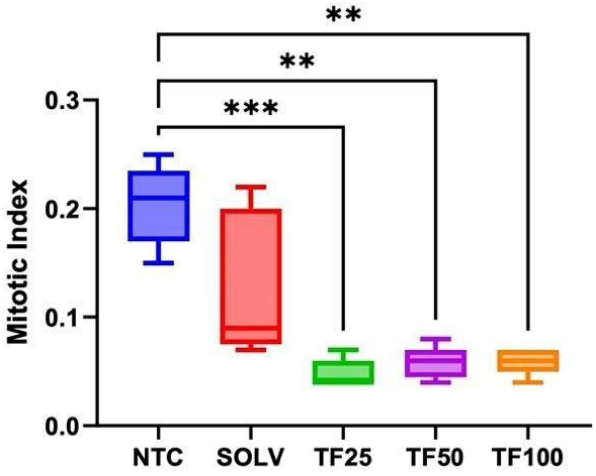

Figure 5 Mitotic index. **= p<0.01; ***= p<0.001.

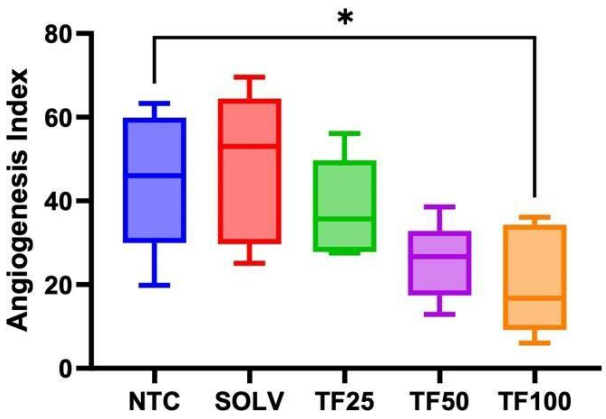

Figure 6 Angiogenesis index. *= p<0.05.

This study investigated the ethanol extract of *T. flagelliforme* in nanoemulsion form as a potential antitumor agent against DMBA-induced skin tumors in rats. Prior to testing, the phytochemical potential of T. flagelliforme was evaluated using toxicity tests. An LC_{50} value below 1000 ppm suggests high bioactivity. Previous studies by Fakri et al. (2020) reported values around 494-555 µg/mL in zebrafish embryos, highlighting considerable toxicity and possible antitumor effects of the nanoemulsion.

Administering antitumor drugs reduced tumor volume and increased body weight in the rats, suggesting an improvement in overall health associated with the dosage of *T. flagelliforme*. The highest dosage (TF100) showed the most significant increase in body weight, supporting the hypothesis that effective tumor suppression enhances general health (Ibrahim et al. 2023).

In DMBA-induced rats, microscopic examination revealed anaplastic sarcoma, squamous cell carcinoma (SCC), myosarcoma, and fibrosarcoma. Anaplastic cells, often found in malignant tumors, can be poorly differentiated (Vail *et al.* 2020). SCC showed variable differentiation, keratin pearls, and perinuclear vacuoles. Myosarcoma exhibited spindle cells in rows, while fibrosarcoma contained spindle cells with eosinophilic granules and collagen-like material (Klopfleisch 2016; Vail *et al.* 2020).

Mitosis is the process by which a cell divides to produce two identical daughter cells. The stages of mitosis include prophase, metaphase, anaphase, and telophase. Elevated and abnormal mitosis indicates genetic damage, a feature commonly found in precancerous and cancerous lesions. Defective mitosis leads to various nuclear abnormalities, such as micronuclei, nucleation, broken egg appearance, pyknotic, and an increased number of abnormal mitotic figures. These abnormal mitotic figures are frequently observed in squamous cell carcinomas (SCCs) (Preeti et al. 2019).

The limitless capacity for mitosis in tumors leads to hyperplasia, driven by self-sufficient growth signals and a lack of response to anti-growth signals (Vail et al. 2020). This study used the mitotic index to evaluate cytotoxicity, revealing that all treatment groups experienced a reduction in mitotic cell counts, signifying the anti-proliferative effects of the nanoemulsion. Angiogenesis, the formation of new blood vessels, is critical for tumor growth, and its regulation involves signaling molecules like vascular endothelial growth factor (VEGF) (Florek *et al.* 2024). The nanoemulsion of *T. flagelliforme* demonstrated anti-angiogenic effects, as increased exposure resulted in decreased angiogenesis, further supporting its potential as an antitumor agent.

All these findings (decreasing tumor size, decreased mitosis, decreased angiogenesis) showed that *T. flagelliforme* ethanol extract nanoemulsion effectively inhibits the growth of the tumor. The presence of alkaloids, flavonoids, terpenoids, and steroids with antioxidant properties in *T. flagelliforme* nanoemulsion likely contributed to inhibiting cell proliferation and inducing tumor cell apoptosis (Rostantinata *et al.* 2018). Specifically, flavonoids like isovitexin and vitexin promote apoptosis and inhibit cell proliferation via various signaling pathways. Vitexin's action involves downregulation of Bcl-2 and upregulation of caspase-3 and caspase-9, while also inducing autophagy through Hsp90 activation (He *et al.* 2016). Stigmasterol, a steroid compound, regulates cell death through the PI3K/Akt pathway and modulates cyclin proteins to inhibit proliferation (Zhang *et al.* 2022). Tannin and flavone compounds are reported to exhibit anti-angiogenesis activity in vitro and in ovo (Priosoeryanto *et al.* 2020).

Nanoparticle delivery systems, such as nanoemulsions, enhance the targeting of tumor cells, overcome drug resistance, and improve the pharmacokinetics of therapeutic agents (Dadwal *et al.* 2018; Palazzolo *et al.* 2018). The particle size and distribution of the *T. flagelliforme* nanoemulsion suggest it has the potential for effective delivery of bioactive compounds, improving their solubility and absorption while potentially reducing required dosages (Ningsih *et al.* 2017; Rahmasari *et al.* 2021).

In conclusion, the ethanol extract nanoemulsion of *T. flagelliforme* exhibits significant antitumor activity by reducing tumor size and contains bioactive compounds with anti-proliferative and anti-angiogenic properties. This study underscores the potential of this nanoemulsion as a novel therapeutic strategy in antitumor treatments.

"The authors report no conflicts of interest in this work".

REFERENCES

Dadwal A, Baldi A, Narang RK. 2018. Nanoparticles as carriers for drug delivery in cancer. Artif Cells Nanomed Biotechnol 46(2):295–305.

El Yaagoubi OM, Lahmadi A, Bouyahya A, Filali H, Samaki H, El Antri S, Aboudkhil S. 2021. Antitumor effect of *Inula viscosa* extracts on DMBA-induced skin carcinoma are mediated by proteasome inhibition. Biomed Res Int. 2021 Mar:6687589.

Fakri F, Idrus LS, Iskandar MA, Wibowo I, Adnyana IK. 2020. Acute toxicity of keladi tikus (*Typhonium flagelliforme* (Lodd.) Blume) ethanol extract on zebrafish (*Danio rerio*) embryo in vivo. Indonesian J Pharm 31(4):297–304.

Florek K, Mendyka D, Gomułka K. 2024. Vascular endothelial growth factor (VEGF) and its role in the cardiovascular system. Biomedicines 12:1055.

Harborne JB. 1996. Method of Phytochemistry. 2nd ed. Bandung: ITB Press.

Harwansh RK, Deshmukh R, Rahman MA. 2019. Nanoemulsion: Promising nanocarrier system for delivery of herbal bioactives. J Drug Deliv Technol 51:224.

He M, Min JW, Kong WL, He XH, Li JX, Peng BW. 2016. A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia 115:74–85.

Ibrahim MY, Hashim NM, Omer FAA, Abubakar MS, Mohammed HA, Salama SM, Jayash SN. 2023. Potential antitumor effect of α-mangostin against

rat mammary gland tumors induced by LA7 Cells. Int J Mol Sci 24(12):10283.

Klopfleisch R. 2016. *Veterinary Oncology:A Short Textbook*. Gewerbestrasse: Springer.

Lewandowska AM, Rudzki M, Rudzki S, Lewandowski T, Laskowska B. 2019. Environmental risk factors for cancer - review paper. Ann Agric Environ Med 26(1):1–7.

Mao X, Cai Y, Long S, Perez-Losada J, Mao J, Chang H. 2023. Pan-cancer evaluation of clinical value of mitotic network activity index (MNAI) and its predictive value for immunotherapy. Front Oncol 13:1178568.

Mathewos M, Demissie T, Fasseha H, Yirgalem M. 2020. Histological, cytological characteristics and treatment options on common skin tumors of domestic animals: A review. Int J Rec Biotechnol 8(1):1–24.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 45:31–34.

Muti'ah R, Griana TP, Ula QN, Andyarto Y. 2016. The effect of *Calotropis gigantea* leaves extract on fibrosarcoma growth and caspase 3 expression. In J Pharm Clin 8(3):167–171.

Ningsih N, Yasni S, Yuliani S. 2017. Sintesis nanopartikel ekstrak kulit manggis merah dan kajian sifat fungsional produk enkapsulasinya [Synthesis of red mangosteen peel extract nanoparticles and study of the functional properties of their encapsulated products]. JTIP 28(1):27–35. In Indonesian Language.

Ostertag F, Weiss J, McClements DJ. 2012. Low-energy formation of edible nanoemulsions: Factors influencing droplet size produced by emulsion phase inversion. J Colloid Interface Sci 388(1):95–102.

Palazzolo S, Bayda S, Hadla M, Caligiuri I, Corona G, Toffoli G, Rizzolio F. 2018. The clinical translation of organic nanomaterials for Cancer therapy: A focus on polymeric nanoparticles, micelles, liposomes and exosomes. Curr Med Chem 25(34):4224–4268.

Prakash S, Singh P, Nagayach P, Singh K, Bharti R, Prakash G. 2022. Angiogenic index as a measure of angiogenesis in prostate cancer and its correlation with gleason grade and score. J Clin Diagn Res 16(12):EC05–EC09.

Preeti S, Vijay K, Ganesh S, Anshu S, Ankita S. 2019. Significance of the apoptotic index, mitotic index, and turnover index in premalignant and malignant squamous cell lesion of the oral cavity: How much is their diagnostic and prognostic role?. J Sci Soc 46(2):49–52.

Preeti, Sambhakar S, Malik R, Al Harrasi A, Rani C, Saharan R, Kumar S, Geeta, Sehrawat, R. 2023. Nanoemulsion: An emerging novel technology for improving the bioavailability of drugs. Scientifica (Cairo) 2023 Oct:6640103.

Priosoeryanto BP, Rostantinata R, Harlina E, Nurcholis W, Ridho R, Sutardi LN. 2020. In vitro antiproliferation activity of *Typhonium flagelliforme* leaves ethanol extract and its combination with canine interferons on several tumor derived cell lines. Vet World 13(15):931–939.

Priosoeryanto BP. 1994. Morphological and Biological Studies of Tumor in Domestic Animals [dissertation]. Yamaguchi: Yamaguchi University.

Rachmawati AS. 2020. Prevalensi kanker di Rumah Sakit Jasa Kartini Kota Tasikmalaya tahun 2018 [Prevalence of cancer at Jasa Kartini Hospital, Tasikmalaya City, in 2018]. JKKI 16(1):119–126. Indonesian. In Indonesian Language.

Rahmasari D, Rosita N, Soeratri W. 2021. Physicochemical characteristics, stability, and irritability of nanostructured lipid carrier system stabilized with different surfactant ratios. JFIKI 9(1):8–16.

R**ostantin**ata R, Priosoeryanto BP, Harlina E, Nurcholis W, Sutardi LN, Ridho R. 2018. Antiproliferation activity of keladi tikus (*Typhonium flagelliforme*) leaves ethanol extract on MCA-B1 and MCM-B2 tumor – derived cell lines in vitro. *Proceedings of the 20th FAVA CONGRESS & the 15th IVMA Veterinary Scientific Conference (KIVNAS); 2018 Nov 1–3;* Bali, Indonesia. Jakarta: Indonesian Veterinary Medical Association. In Indonesian Language.

Ruple A, Bonnett BN. 2019. Withrow and MacEwen's Small Animal Clinical Oncology. 6th ed. St. Louis: W.B. Saunders.

Sianipar NF, Yuliani S, Purnamaningsih R, Assidqi K, Abbas BS. 2020. The physical and chemical analysis of nanoemulsion from extract rodent tuber mutant plant (*Typhonium flagelliforme* Lodd). IOP Conf Ser Earth Environ Sci 439:012005.

Vail DM, Thamm DH, Liptak JM. 2020. Withrow and MacEwen's Small Animal Clinical Oncology. 6th ed. St. Louis: Elsevier.

Veselov VV, Nosyrev AE, Jicsinszky L, Alyautdin RN, Cravotto G. 2022. Targeted delivery methods for anticancer drugs. *Cancers (Basel)*. 14(3):622.

Wang X, Zhang H, Chen X. 2019. Drug resistance and combating drug resistance in cancer. Cancer Drug Resist 2:141–160.

Zhang X, Wang J, Zhu L, Wang X, Meng F, Xia L, Zhang H. 2022. Advances in stigmasterol on its anti-tumor effect and mechanism of action. Front Oncol 12:1101289.