Research Article

Evaluation of Biological Activities of *Eryngium foetidum* Leaf Extracts under Different Drying Temperatures and Extraction Solvents

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Abstract | *Eryngium foetidum*, known as 'Ketumbar Jawa' in Malay, is a traditional medicinal herb recognized for its antioxidant, antiproliferative, and antimicrobial properties. Despite its traditional use, limited scientific data exist on the bioactivities of locally grown *E. foetidum*. This study aimed to assess the biological activities of *E. foetidum* leaf extracts obtained using different drying temperatures (40˚C and 60˚C) and extraction solvents (ethanol and methanol). The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) assay, Ferric Reducing Antioxidant Power (FRAP) assay, and 2,2`-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization (ABTS) assays. Antimicrobial properties were assessed using the disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the antiproliferative effects on human breast adenocarcinoma (MCF-7) cells. Results were expressed as mean ± SD (n=3) and analyzed using one-way ANOVA with significance at p <0.05. Results showed that the methanol (MeOH:40˚C) extract exhibited the strongest antioxidant activity, with IC_{50} values of 542, 72.75, and 322.8 µg/mL for DPPH, FRAP, and ABTS respectively. Antimicrobial activity was only significant against *S. aureus*, with ethanol (EtOH:40˚C) and MeOH:40˚C extracts demonstrating higher inhibition zones at specific concentrations. The EtOH:40˚C extract showed the highest antiproliferative effect against MCF-7 cells (IC_{50}: 394 µg/mL), comparable to MeOH:40˚C. Lower drying temperatures were more effective in preserving bioactive compounds, enhancing both antioxidant and antiproliferative activities. In conclusion, *E. foetidum* leaf extracts, dried at 40˚C, possess enhanced antioxidant and antiproliferative activities, suggesting their promising potential for therapeutic applications

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Introduction

Malaysia's diverse flora has long been central to indigenous medicinal practices, reflecting the region's cultural and ecological richness (Tan *et al.*, 2020). Among these botanical resources, *Eryngium foetidum* L., commonly known as 'Ketumbar Jawa' or spiny coriander, has drawn considerable attention for its dual role in culinary and therapeutic applications. Belonging to the Apiaceae family, the plant is widely used in Southeast Asia and Latin America, where its serrated leaves and pungent aroma are valued in cuisine, while its medicinal properties have been traditionally employed to relieve digestive disorders, fevers, and inflammatory conditions (Hemachandra *et al.*, 2021). Despite the benefits of medicinal plants, heavy metal contamination in soil poses a quality risk that bioremediation can help to address (Al-Jaberi, 2024). Botanically, *E. foetidum* is a perennial herb that typically grows up to 30–40 cm in height, characterized by a basal rosette of elongated, lanceolate leaves with spiny-toothed margins. The leaves, measuring 10–20 cm long, are leathery in texture and emit a strong coriander-like odor when crushed. The plant produces small white to greenish flowers arranged in dense, conical umbels surrounded by spiny bracts, which are distinctive morphological features of the genus (Rodrigues *et al.*, 2022; Hemachandra *et al.*, 2021). It thrives in moist, shaded habitats but can also be cultivated under tropical agricultural conditions, making it accessible both as a culinary herb and a source of medicinal compounds (Rodrigues *et al.*, 2022).

The pharmacological potential of *E. foetidum* is closely linked to its diverse phytochemical composition, which includes essential oils, flavonoids, tannins, alkaloids, and phenolic acids (Rodrigues *et al.*, 2022; Leitão *et al.*, 2023). In particular, polyphenols and flavonoids have been associated with antioxidant, antimicrobial, and anticancer activities (Rodrigues *et al.*, 2022). Antioxidants mitigate oxidative stress by neutralizing free radicals, thereby reducing the risk of chronic diseases, while antiproliferative and antimicrobial activities position *E. foetidum* as a candidate for cancer prevention and infectious disease management (Sruthi and Danya, 2021).

Despite these promising findings, most previous studies have been limited to single extraction methods or narrow sets of bioassays. For instance, Hernández-López *et al.* (2023) examined the ethnobotany, phytochemistry, and biological activities of Eryngium species in Mexico but did not evaluate how post-harvest processing influences its bioactivity. Furthermore, little is known about the combined influence of solvent type and drying temperature on the phytochemical profile and biological activity of *E. foetidum*. Since extraction solvents such as ethanol and methanol can yield different classes and concentrations of compounds (Sasidharan *et al.*, 2010; Ndiaye *et al.*, 2024), while drying conditions strongly affect the stability of bioactive metabolites (Gąsecka *et al.*, 2020). The study of baobab seed oil revealed that different extraction processes modified its chemical composition and reduced sensitive antioxidants, demonstrating the impact of processing on bioactive compounds (Ndiaye *et al.*, 2024). Similarly, in *E. foetidum*, drying and extraction methods are critical, necessitating a systematic approach to identify the best conditions for preserving phytochemicals.

To address this gap, the present study investigates the effects of drying temperature (40 °C and 60 °C) and extraction solvent (ethanol and methanol) on the phytochemical composition and biological activities of Malaysian-grown *E. foetidum* leaves. By assessing antioxidant, antiproliferative, and antimicrobial properties in a unified experimental framework, this study provides new insights into the therapeutic potential of *E. foetidum* and supports its future development as a source of herbal remedies.

Material and Methods

Extraction of E. foetidum leaves

The fresh mature leaves of *E. foetidum* were collected from Kampung Amir, Alor Lintang, 22200 Besut, Terengganu (5°46'36.6"N 102°32'08.1" E). The fresh and healthy leaves of *E. foetidum* were washed with running tap water to clean all the dirt on the surface of the leaves and rinsed with tap water. The leaves were chopped into smaller pieces and divided into two group of samples that dried in different drying temperature (40°C and 60°C) for 72 hours in incubator. The dried samples of each temperature were ground into a coarse powder by using a blender. Then, the samples of each temperature were soaked with ethanol (EtOH) in a conical flask and stirred. The mixture was left for three days. The samples were stirred with a spoon daily. After three days, the extracts were filtered using filter paper. The filtered

samples were concentrated by using rotary evaporator to obtain the ethanol crude extracts with different drying temperature and then stored at 4°C. The process was repeated by using methanol (MeOH) as extraction solvent to obtain methanol crude extracts with different drying temperature. At the end of the extraction process, four *E. foetidum* leaf extracts with different combination of drying temperature and extraction solvent were obtained as follow: Ethanol extraction at 40°C (EtOH:40°C), Ethanol extraction at 60°C (EtOH:60°C), Methanol extraction at 40°C (MeOH:40°C), and Methanol extraction at 60°C (MeOH:60°C).

Total phenolic content (TPC)

The TPC of the *E. foetidum* leaf extract were determined using Folin-Ciocalteu's colourimetric technique (Zin *et al*., 2018). A stock solution of gallic acid of 1 mg/mL was prepared by dissolving gallic acid in methanol. The standard solution was prepared at six different concentrations: 0, 20, 40, 60, 80 and 100 µg/mL. A total volume of 60 µL of sample or standard was added into an Eppendorf tube, followed by 40 µL of methanol. Approximately 200 µL of Follin Ciocalteu reagent was added and vortexed thoroughly. Then, 800 µL of 7.5 % sodium carbonate (Na_2CO_3) was added and incubated for 2 hours at room temperature. About 200 µL of the prepared mixture was transferred into a 96-well plate. The absorbance was measured using a microplate reader at 765 nm. Three measurements were taken. The gallic acid standard curve was used to calculate the total phenolic content using the following formula, where C is the total phenolic content (mg of GAE / g of plant extract), c is the concentration of gallic acid (mg/mL) established from the standard curve, V is final volume of plant extract, and m is the weight of the plant extract. The results were measured in milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

$$C=cV/m$$

Total flavonoid content (TFC)

The TFC of *E. foetidum* leaves extract was determined by using a previously reported (Zin *et al*., 2018). A stock solution of quercetin at a concentration of 1 mg/mL was prepared by dissolving it in methanol. Subsequently, the stock solution was used to prepare standard solution at six different concentrations: 0, 20, 40, 60, 80 and 100 µg/mL. Then, a mixture was prepared by combining 140 µL of sample extracts or standard, 260 µL methanol, 150 µL 1 M potassium acetate, and 150 µL 10% aluminium chloride. At room temperature, the solution was incubated for 30 minutes. About 200 µL of prepared mixture were transferred into 96 well plate. The absorbance was measured using microplate reader at 415 nm. Three measurements were taken. The quercetin standard curve was used to calculate the total flavonoid content using the following formula, where C is the total flavonoid content (mg of QE / g of plant extract), c is the concentration of quercetin (mg/mL) from the standard curve, V is the final volume of plant extract, and m is the weight of the plant extract. The results were given in milligrams of quercetin equivalents per gram (mg QE/g) of samples extract.

$$C=cV/m$$

2,2-Diphenyl-1-Picry-Hydrazyl-Hydrate (DPPH) Assay

The previously reported method was adopted (Athilah *et al*., 2020). A stock solution of the extract was prepared at a concentration of 5 mg/mL in DMSO. A blank solution comprising DMSO and DPPH was made as a control. Ascorbic acid was served as the standard. In 96-well plates, 150 µL of 0.1 mM DPPH working solution were added to the 50 µL sample or standard of different concentrations: 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/mL. The mixture was thoroughly mixed and left in the dark for 30 minutes. A microplate reader was used to measure the change in absorbency at 517 nm. Three measurements were taken. The percentage of DPPH scavenging activity was calculated as follows where, A_c = absorbance of the control and A_s = absorbance of the sample.

$$Inhibition\ (\%) = [(A_s - A_c) / A_s] \times 100$$

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was determined according to past study method (Athilah *et al*., 2020). A stock solution of the extract was prepared at a concentration of 5 mg/mL in DMSO. A blank solution comprising DMSO and FRAP working solution was made as a control. Ascorbic acid was served as the standard. First solution which is 0.3 M acetate buffer pH 3.6 was prepared by combining 0.16 g sodium acetate and 100 mL 0.28 M glacial acetic acid. The solution pH was adjusted with 1 M NaOH or 1 M HCl. Second solution which is 0.01 M of TPTZ solution was prepared by mixing 0.31 g of TPTZ in 100 mL of 40 mM

HCl. For 0.02 M ferric (III) chloride (solution 3) was prepared by diluting 0.32 $FeCl_3$ with 100 mL distilled water. To make FRAP reagent, solution 1, solution 2 and solution 3 in 10:1:1 ratio was combined and heated in a water bath at 37°C for 30 minutes. 150 µL FRAP reagent were combined with 50 µL sample extract or standard with different concentrations of 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/mL. The absorbance was measured at 593 nm immediately using microplate reader. The percentage of inhibition was calculated with the following equation where As = absorbance of the sample and Ac = absorbance of the control.

Inhibition (%) = $[(A_s - A_s) / A_s] \times 100$

2,2'-azino-bis (3- ethylbenzthiazolin-6-sulfonic acid) (ABTS) assay

ABTS radical scavenging activity was determined according to the past study method (Asraoui *et al.*, 2021). A stock solution of the extract was prepared at a concentration of 5 mg/mL in DMSO. A blank solution comprising DMSO and ABTS working solution was made as a control. Ascorbic acid was served as the standard. The ABTS stock solution was prepared by reacting 7 mM ABTS aqueous solution with 2.45 mM aqueous solution of potassium persulfate ($K_2S_2O_8$) and allowed the mixture to react in the dark at room temperature for 12–16 h. Then the $ABTS^+$ stock solution was adjusted with methanol to obtain an initial absorbance value of 0.7 ± 0.02 at 738 nm. Approximately 50 µL of the extract sample was mixed with 150 µL of $ABTS^+$ diluted solution, and the mixture was incubated for 6 min. A microplate reader was used to measure the change in absorbance at 738 nm. The percentage of inhibition was calculated according to the following equation, where: A_c is the absorbance of the control, and A_s is the absorbance of the sample.

Inhibition (%) = $[(A_c - A_s)/A_c] \times 100$

Discuss diffusion method

Disc diffusion method was determined according to previously reported method (Dutta *et al.*, 2017). The bacterial strains used (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922) were obtained from the Microbiology Laboratory, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA). *S. aureus* and *E. coli* suspension cultures were prepared in MH broth and adjusted to 0.5 McFarland turbidity standard. The surface of the MH agar plate was streaked with the bacterial inoculum. Sterile filter paper discs, with diameter 6 mm were placed on the MH agar surface using sterile forceps and then impregnated with 20 µL of the *E. foetidum* leaf extracts with five different concentrations (20, 40, 60, 80, 100 mg/mL). Disc impregnated with DMSO was used as negative control. For positive control, antibiotic Gentamicin and Ciprofloxacin were used. The plates were incubated at the 37°C for 24 hours. The diameter of the clear zones of inhibition around each disc was measured.

Cell culture

The human breast adenocarcinoma cell line (MCF-7) was used for this study and provided by the UniSZA. The cells were grown in a T25 flask supplied with Roswell Park Memorial Institute (RPMI) growth media, containing 10% fetal bovine serum (FBS). The cells were incubated at 37°C in a 5% CO_2 incubator. The cells were grown until confluence and subculture at 2 to 3 days intervals.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The effects of the *E. foetidum* leaf extracts on cell proliferation were measured by MTT assay based on the ability of viable cells to convert tetrazolium salt into purple formazan. Briefly, 100 µL of 2×10^5 cells/mL of cells were seeded into 96-well plates overnight. The cells were then treated with 100 µL of different concentrations *E. foetidum* leaf extracts ranging from 0 to 2000 µg/mL. The cells also were treated with different concentration of positive control Etoposide ranging from 0 to 100 µg/mL. The treated cells were incubated in an incubator at 37°C for 72 hours in a 5% CO_2 incubator. After incubation time, 20 µL of 5 mg/mL MTT reagent were added and incubated for 4 hours in the dark to allow the mitochondrial dehydrogenases of viable cells cleaved the tetrazolium ring and yield purple formazan crystal which is insoluble in an aqueous solution. After 4 hours incubation time, the half of the media was removed and 100 µL of DMSO were added to the wells before incubating for another 10 minutes. The absorbance was measured at 570 nm using a microplate reader. The half-maximal inhibitory concentration (IC_{50}) value was computed from a graph of percentage cell viability versus the concentration of the extracted sample with the help of software GraphPad Prism 9.

Morphological observation

The cells were seeded in a 12-well plate at a concentration of 2 × 10^5 cells/mL and incubated overnight in a humidified 5% CO_2 incubator at 37°C. The cells were than treated at moderate dose (IC_{50}) of E. foetidum leaf extracts or control Etoposide before being incubated at 24, 48, and 72 hours. The untreated cells were used as negative control for comparison. The morphological changes of the treated and untreated cells were be observed using a phase contrast microscope.

Statistical analysis

The statistical analysis of data was performed by using software GraphPad Prism version 9. All experiments were performed in triplicate and each data represents mean ± standard deviation (SD). Comparisons of the group were done using one-way or two-way Analysis of Variance (ANOVA), followed by the Tukey post-test for multiple comparisons (* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns for no significant). One-way ANOVA analysis was performed for data of TPC, TFC, DPPH, FRAP, ABTS and MTT assay, meanwhile two-way ANOVA was performed for data of antimicrobial assessment.

Results and Discussion

Total phenolic content (TPC) and total flavonoid content (TFC) of E. foetidum leaf extracts

The evaluation of biological activities of *E. foetidum* leaf extracts often involves assessing the total phenolic and flavonoid content. Based on Figure 1, the highest TPC was recorded in MeOH:40°C (51.23 mg

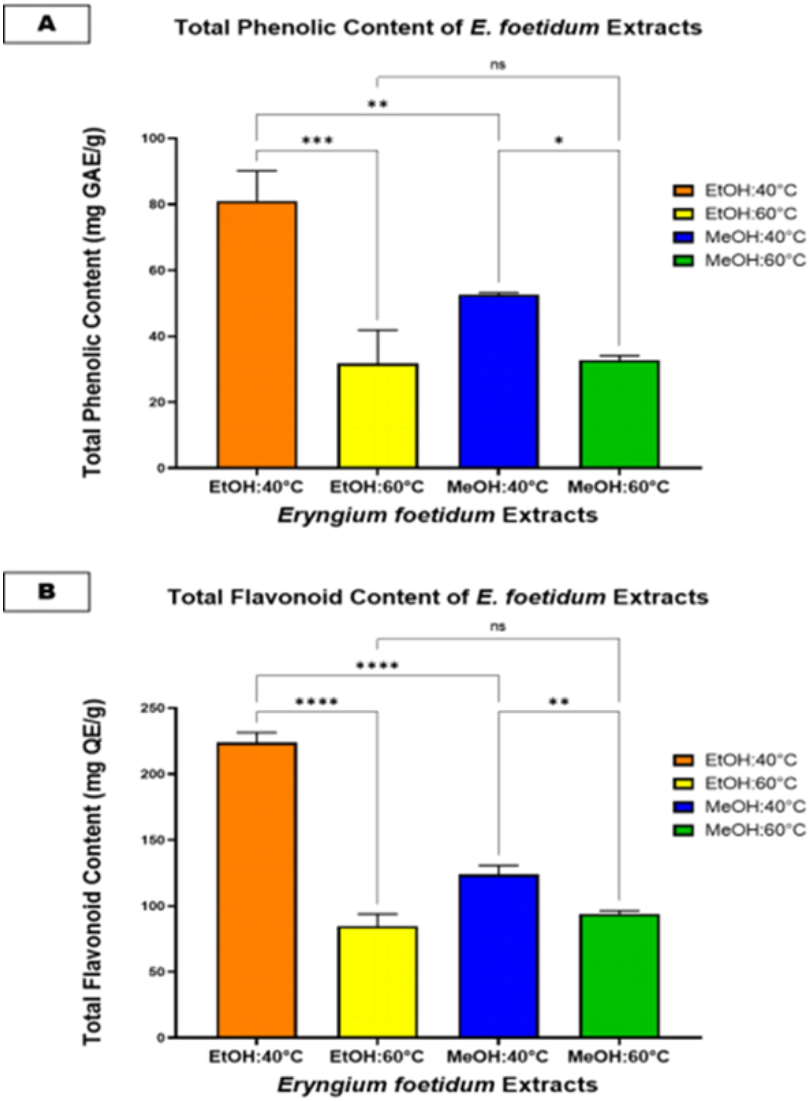

Figure 1: *The total phenolic and flavonoid content of E. foetidum leaf extracts. (A) total phenolic content E. foetidum leaf extracts. (B) total flavonoid content E. foetidum leaf extracts. Data represents mean ± SD, n = 3. Comparisons of the group were done using one-way ANOVA, followed by the tukey post-test for multiple comparisons (* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns for no significant).*

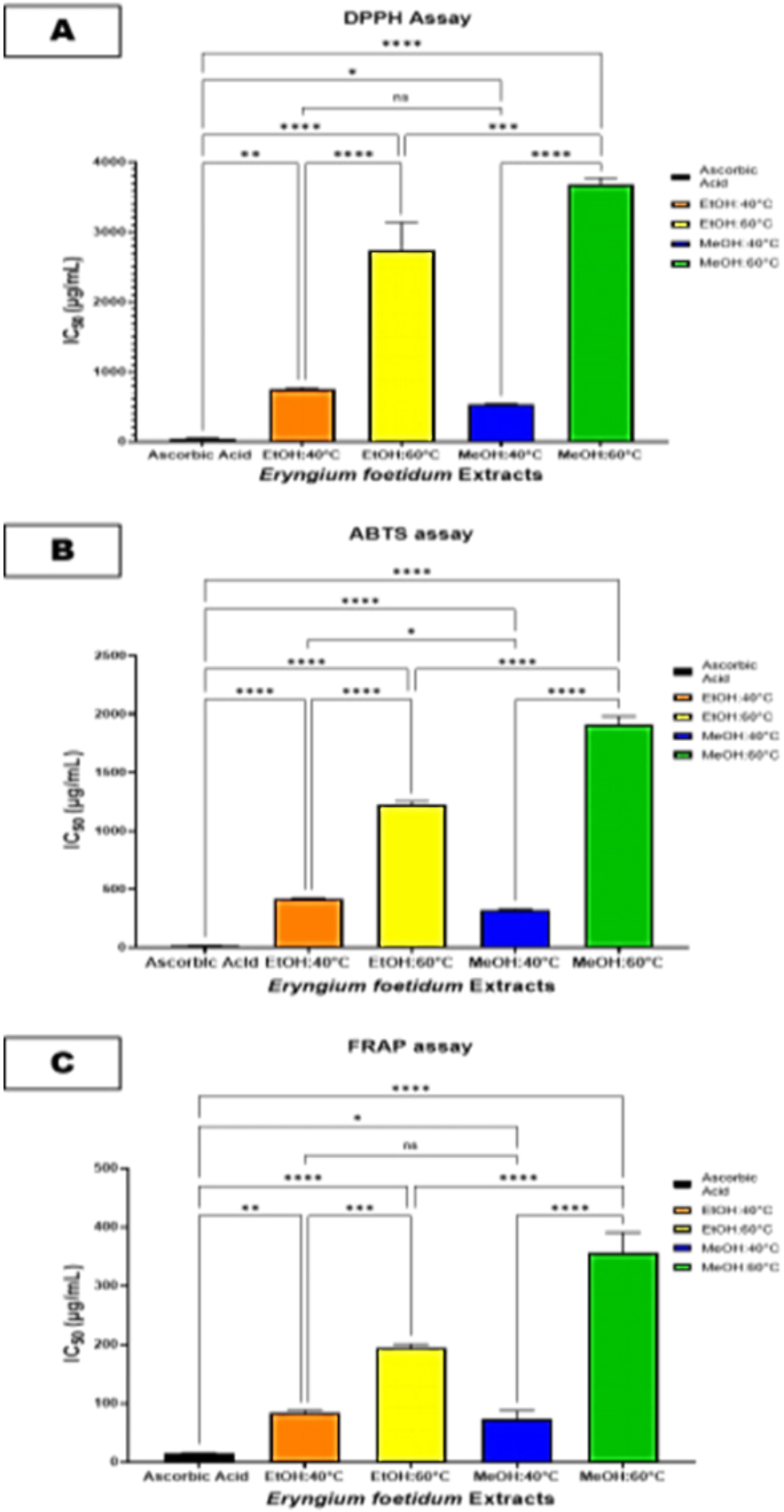

Figure 2: *Antioxidant activity of E. foetidum leaf extracts. (A) IC_{50} DPPH scavenging activity of E. foetidum leaf extracts compared to standard ascorbic acid. (B) IC_{50} ABTS scavenging activity of E. foetidum leaf extracts compared to standard ascorbic acid. (C) IC_{50} ferric ion reducing activity of E. foetidum leaf extracts compared to standard ascorbic acid of FRAP assay. Data represents mean ± SD, n = 3. Comparisons of the group were done using one-way ANOVA, followed by the Tukey post-test for multiple comparisons (* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns for no significant).*

GAE/g), which was significantly higher ($p < 0.05$) than EtOH:40°C (46.12 mg GAE/g) and both 60°C extracts. Similarly, TFC was highest in MeOH:40°C (32.45 mg QE/g), followed by EtOH:40°C (29.88 mg QE/g), with both values significantly higher ($p < 0.05$) than the 60°C treatments. At lower drying temperature (40°C), EtOH extract shows higher TPC & TFC values than MeOH extract. The choice of solvent can significantly affect the TPC and TFC in plant extracts. Different solvents have varying polarities and extraction efficiencies, which can influence the extraction of phenolic and flavonoid compounds. The effectiveness of ethanolic extract may be attributed to its ability to extract the specific flavonoids and phenolic compounds present in *E. foetidum* (Dirar *et al.*, 2019). Within the same extraction solvent, EtOH:40°C extract has more TPC & TFC values than EtOH:60°C extract. The results are also similar with methanol solvent, where MeOH:40°C extract has more TPC & TFC values than MeOH:60°C extract. This is because elevated drying temperatures may lead to the degradation of heat-sensitive compounds, contributing to a reduction in both TPC and TFC (ElGamal *et al.*, 2023).

In this study, the *E. foetidum* leaf dried at 40°C drying temperature and extracted using ethanol appears to be the most effective combination for obtaining extracts with high phenolic and flavonoid concentrations. Several studies have identified the presence of various phenolic compounds in *E. foetidum* leaf extracts. One study found that the extract exhibited a high concentration of phenolic compounds, including total tannins and total flavonoids (Thi *et al.*, 2020). Another study identified six phenolic compounds in *E. foetidum* leaf extracts, with chlorogenic acid being the major compound (Leitão *et al.*, 2023). Additionally, the study mentioned that the mixture of water and ethanol yielded the highest contents of flavonols in the extract, represented by quercetin glucuronide (Leitão *et al.*, 2023).

Antioxidant activity of e. foetidum leaf extracts

Antioxidant activity refers to the ability of a substance to neutralize or inhibit the oxidation of other molecules, thereby preventing or reducing the damage caused by free radicals and reactive oxygen species (ROS) (Leitão *et al.*, 2023). *E. foetidum*, an aromatic and medicinal herb, has been found to exhibit strong antioxidant activity in its essential oils and leaf extracts (Thi *et al.*, 2020). In this study, DPPH, ABTS and FRAP assays were performed to measure the antioxidant activity of *E. foetidum* leaf extracts.

According to Figure 2, the IC_{50} results for all antioxidant assays followed a pattern consistent with the phytochemical data. Extracts obtained with methanol demonstrated significantly stronger radical scavenging and reducing activity compared to ethanol extracts. Among drying conditions, 40 °C extracts exhibited higher antioxidant activity than those dried at 60 °C across all assays. Standard ascorbic acid had the lowest IC_{50} value than all *E. foetidum* leaf extracts. The lower the IC_{50} value, the higher the antioxidant activity of the tested sample. The MeOH:40°C extract exhibited the strongest antioxidant activity with IC_{50} values of 542 µg/mL (DPPH), 72.75 µg/mL (FRAP), and 322.8 µg/mL (ABTS), all significantly lower ($p < 0.05$) than other extracts, indicating higher antioxidant potential. The results indicate that the MeOH:40°C exhibited the lowest IC_{50} value among all the extracts followed by EtOH: 40°C. On the other hand, the MeOH: 60°C showed the highest IC_{50} value, indicating relatively lower antioxidant activity compared to the other extracts. This suggests that the antioxidant activity of *E. foetidum* is influenced by the choice of solvent and extraction temperature, with the 40°C condition showing higher efficacy compared to 60°C. Ethanol and methanol are commonly used solvents due to their ability to extract a broad spectrum of phytochemicals with antioxidant properties. Methanol extracts generally exhibit superior antioxidant activity compared to ethanol extracts (Dirar *et al.*, 2019). But according to DPPH and FRAP results, MeOH and EtOH extract at 40°C temperature shows no significant different of antioxidant scavenging activity. This indicates that both solvent at 40°C are equally effective in extracting antioxidant compounds *E. foetidum* plant.

The influence of drying temperature on the antioxidant activity of plant extracts has also been investigated in previous studies. Temperature can affect the stability of bioactive compounds, potentially leading to the degradation of antioxidant molecules. In this study, the lower IC_{50} value of MeOH and EtOH at 40°C may be due to the preservation of heat-sensitive antioxidants at lower temperatures (Hernández-Bolio *et al.*, 2019). Elevated drying temperatures may lead to the degradation of heat-sensitive compounds, contributing to a reduction in both TPC and TFC

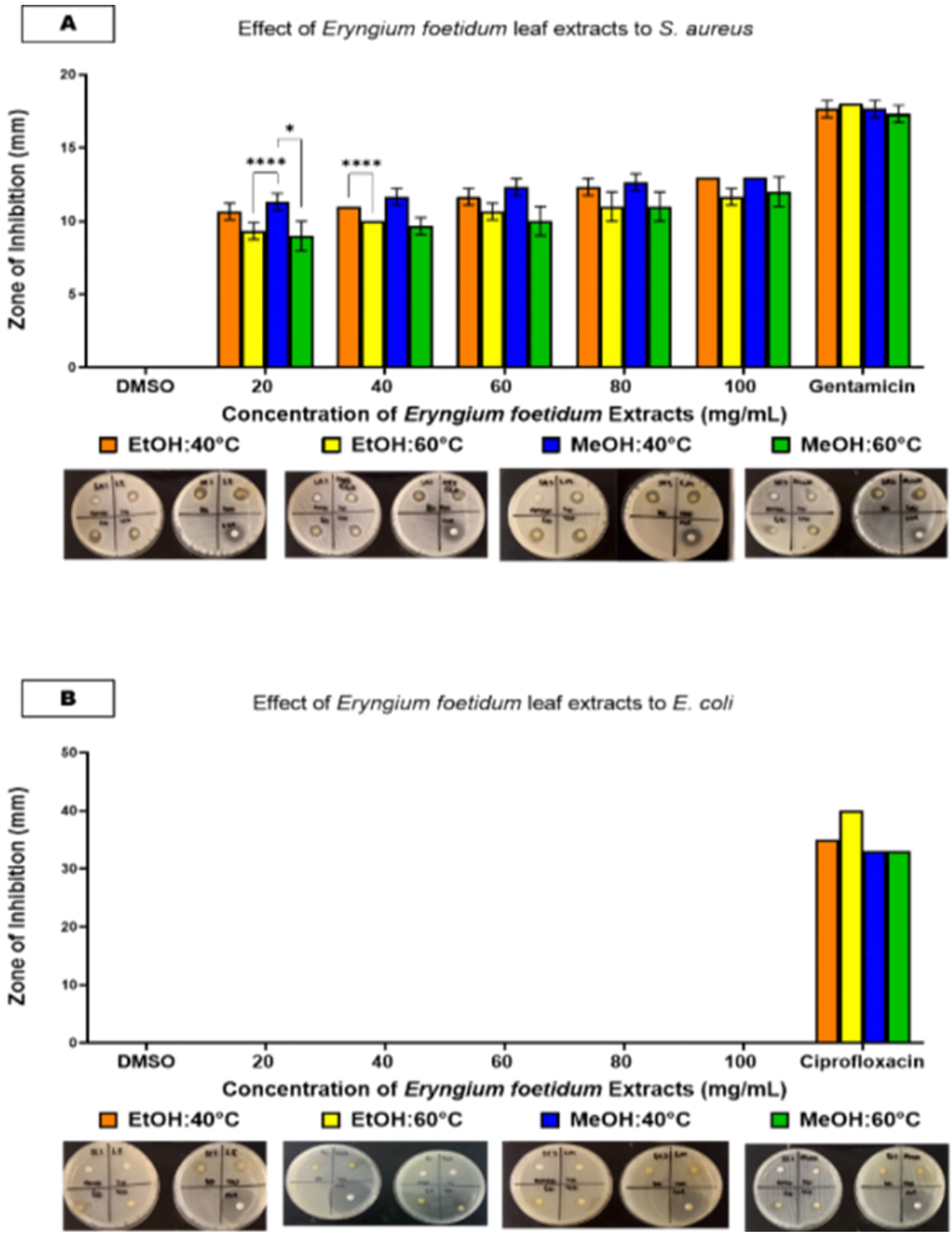

Figure 3: *Antimicrobial activity of E. foetidum leaf extracts.* ***(A)*** *The effect of different concentration of E. foetidum leaf extracts to the growth of S. aureus. (B) The effect of different concentration of E. foetidum leaf extracts to the growth of E. coli. Data represents mean ± SD, n = 3. Comparisons of the group were done using two-way ANOVA, followed by the Tukey post-test for multiple comparisons (* p<0.05; **** p<0.0001).*

(ElGamal *et al.*, 2023). Likewise, alcoholic extracts of *Artemisia absinthium* demonstrated strong bioactivity due to the retention of phenolic antioxidants, emphasizing the critical role of solvent and temperature in preserving antioxidant potential in medicinal plants (Alani and Kawan, 2024). Compared with the standard ascorbic acid, it is evident that the leaf extracts of *E. foetidum* possess antioxidant activity, as indicated by the IC_{50} values. However, the standard ascorbic acid exhibited a significantly lower IC_{50} value, emphasizing its stronger antioxidant potential compared to the *E. foetidum* extracts.

Antimicrobial Activity of E. foetidum Leaf Extracts

The disc diffusion method was used in this study to evaluate the antimicrobial activity of *E. foetidum* leaf extracts against *Staphylococcus aureus* and *Escherichia coli*. The disc diffusion method is a widely employed technique for assessing the antimicrobial susceptibility of microorganisms to various substances, including plant extracts. Based on the antimicrobial results in Figure 3, all *E. foetidum* extracts displayed significant antimicrobial activity against *S. aureus* and no notable antimicrobial activity was observed against *E. coli*. According to Figure 3(A), the EtOH:40°C extract exhibited a zone of inhibition of 15.2 mm, which was significantly higher ($p < 0.05$) than the EtOH:60°C extract (13.6 mm) at a concentration of 40 mg/mL. Similarly, the MeOH:40°C extract showed a zone of inhibition of 14.8 mm, significantly higher ($p < 0.05$) than the MeOH:60°C extract (12.9 mm) at a concentration of 20 mg/mL. Lower drying temperatures (40°C) likely preserve more bioactive compounds responsible for antimicrobial activity. Heat-sensitive antimicrobial compounds may degrade at higher temperatures, resulting in reduced effectiveness (Hernández-Bolio *et al.*, 2019). The treatment with fenugreek seed extract led to increased phenolic accumulation and biological effectiveness in date palm seedlings, thereby providing additional support for the critical role of extraction and processing conditions in preserving phytochemical bioactivity (Hamza and Almansour, 2024).

Within the same temperature, EtOH and MeOH extract did not shows any significant difference ($p > 0.05$) in inhibiting the growth of *S. aureus*. As the concentration of extracts increased, all *E. foetidum* extracts also showed no significant difference ($p > 0.05$) between each other, indicating that different solvents and drying temperatures do not affect the antimicrobial activity of extracts against *S. aureus*. The antimicrobial activity of *E. foetidum* against *S. aureus* has been reported in several studies. The ethyl acetate extract of *E. foetidum* showed strong antimicrobial activity, and the extract from *E. foetidum* leaves exhibited an average of 23 mm zone inhibition against *S. aureus* (Lingaraju *et al.*, 2016). Additionally, the methanolic extract of *E. foetidum* showed high effective antimicrobial activity against *S. aureus* (Dalukdeniya and Rathnayaka, 2017; Parham *et al.*, 2020). The absence of inhibitory effects of *E. foetidum* leaf extracts on *E. coli* suggests a specific antimicrobial activity against Gram-positive bacteria, such as *S. aureus*, rather than a broad-spectrum effect. This differential response could be attributed to variations in the cell wall structure and composition between Gram-positive and Gram-negative bacteria. Based on past study, a study on the antibacterial activity of aqueous and methanol extracts of *E. foetidum* showed mild to moderate antibacterial activity against *S. aureus*, but no activity against *E. coli* was observed (Dutta *et al.*, 2017). However, another study reported that the extract from *E. foetidum* leaves exhibited an average of 20 mm zone inhibition against *E. coli* and an average of 23 mm zone inhibition against *S. aureus* (Menchavez *et al.*, 2018).

Antiproliferative activity of E. foetidum leaf extracts against human breast adenocarcinoma cells line (MCF-7)

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to evaluate the effect of *E. foetidum* leaf extracts against human breast adenocarcinoma cells line (MCF-7). MTT assay is a standard calorimetric assay that used to measure the activity of enzyme That reduced MTT to formazan after cells being treated with a plant extract. Figure 4 displays the IC_{50} values, indicating the concentration of E. foetidum leaf extracts or etoposide required to reduce the cell population by 50%, while Table 1 provides a summary of these IC_{50} values for the different treatments. The order of the IC_{50} value for each sample is as follow: Etoposide (4.6 µg/mL) < EtOH:40°C (394.6 µg/mL) < MeOH:40°C (421.3 µg/mL) < EtOH:60°C (487.2 µg/mL) < MeOH:60°C (502.7 µg/mL). Comparison between *E. foetidum* leaf extracts shows that the EtOH: 40°C extract exhibits the lowest IC_{50} value at 394.6 ± 109.80 µg/mL, indicating a relatively higher antiproliferative activity compared to other extraction conditions. However, there is no significant difference ($p > 0.05$) between EtOH:40°C and MeOH:40°C, indicating that both solvents at 40°C are equally effective in inhibiting MCF-7 cell proliferation. In contrast, the EtOH:60°C and MeOH:60°C extracts demonstrate higher IC_{50} values ($p < 0.05$), suggesting a reduced cytotoxic effect on MCF-7 cells under these conditions. The observed variations in IC_{50} values among different extraction conditions may be attributed to the distinct composition of bioactive compounds extracted under varying solvent types and temperatures. For both EtOH and MeOH extracts, the 40°C condition has a lower IC_{50} than the 60°C condition, indicating that a lower drying temperature

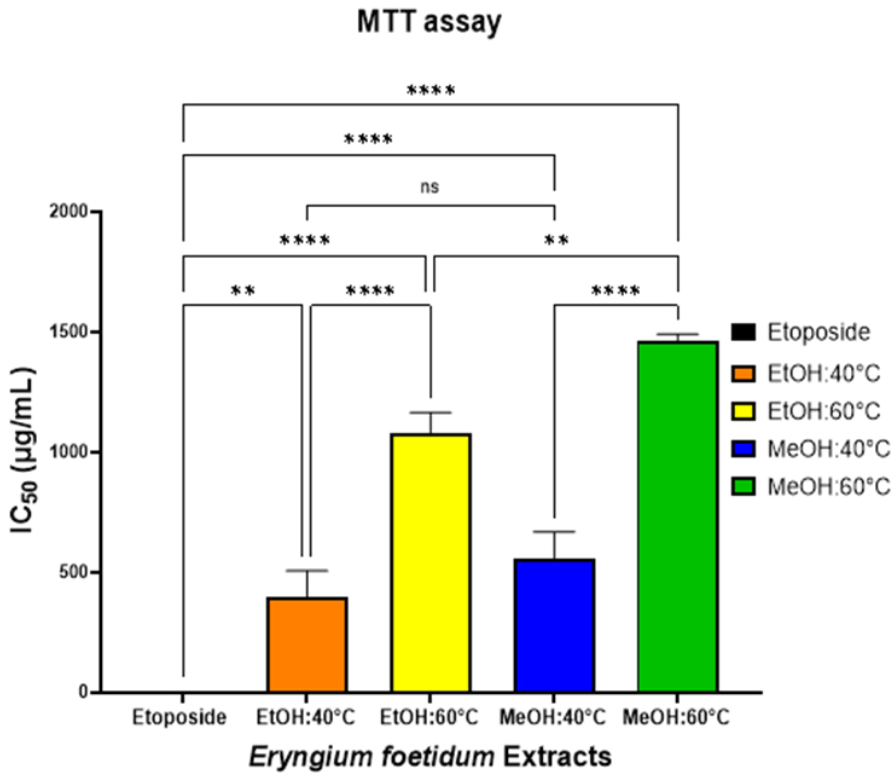

Figure 4: *The IC_{50} value of E. foetidum leaf extracts compared to Etoposide for MTT assay. Data are represented as mean ± SD, n = 3. Comparisons of the group were done using one-way ANOVA, followed by the Tukey post-test for multiple comparisons (** p<0.01; *** p<0.001; **** p<0.0001; ns for no significant).*

is more effective in inhibiting MCF-7 cell proliferation. Lower drying temperatures (40°C) appear to be more favourable for preserving bioactive compounds that exhibit cytotoxicity against MCF-7 cells. Conversely, the higher IC_{50} values for the EtOH: 60°C and MeOH: 60°C extracts may indicate a suboptimal extraction of bioactive compounds or alterations in their composition under prolonged exposure to elevated temperatures. Elevated drying temperatures may lead to the degradation of heat-sensitive compounds, contributing to a reduction in biological activities, including antiproliferative activity (ElGamal *et al.*, 2023). The Etoposide control, a standard chemotherapeutic agent, exhibits a remarkably low IC_{50} value of 4.6 ± 0.38 µg/mL, reaffirming its potent antiproliferative effect as a positive control. The comparison with Etoposide underscores the potential of *E. foetidum* leaf extracts to influence MCF-7 cell viability, although at higher concentrations. Several research have been conducted to test the antiproliferative activity of *E. foetidum* and its constituents. For example, in 2021, Sruthi and Danya studied the cytotoxic activity of the methanolic extract of *E. foetidum* leaves and discovered that it demonstrated considerable cytotoxicity against MCF-7 (Sruthi and Danya, 2021). Another study established the anticancer potential of *E. foetidum* essential oil against human prostate adenocarcinoma cell line (PC-3) (Chandrika *et al.*, 2016). The *E. foetidum* leaf extract exhibited antitumor activity against Ehrlich Ascites Carcinoma in mice, demonstrating therapeutic relevance both *in vitro* and *in vivo* (Ismail *et al.*, 2025).

Table 1: *The IC_{50} of E. foetidum leaf extracts and etoposide for MTT assay. Data are represented as mean ± SD, n = 3.*

Sample	IC_{50} (µg/mL)
EtOH:40°C	394.6 ± 109.80
EtOH:60°C	1079.0 ± 85.91
MeOH:40°C	554.6 ± 112.90
MeOH:60°C	1462.0 ± 27.44
Etoposide control	4.6 ± 0.38

The microscopic examination was performed to observe the effect of IC_{50} dose of *E. foetidum* leaf extracts to the MCF-7 cells after 24, 48 and 72 hours. This to understand time-dependent responses of *E. foetidum* leaf extracts against MCF-7 cells. No dose or untreated MCF-7 cells were served as negative control that representing the normal and healthy state of these breast cancer cells. The cells also were

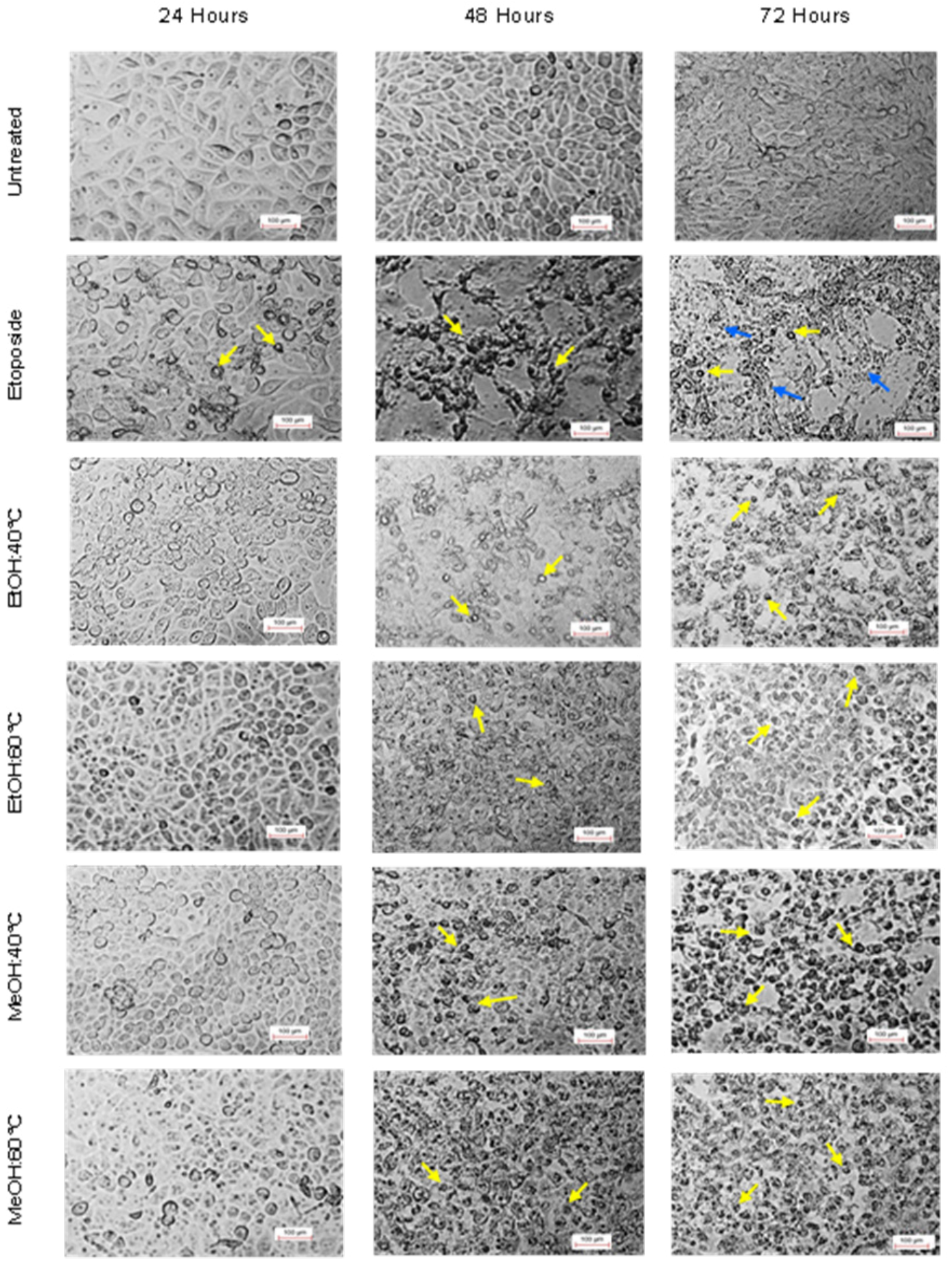

Figure 5: *The microscopic observation on MCF-7 cells after treated with IC_{50} dose of E. foetidum leaf extracts for 72 hours. Yellow arrow shows rounded cell and detaches from the culture flask. Blue arrow shows apoptosis bodies.*

treated with Etoposide and act as positive control that show the true morphological and density changes of the cells after treatment. Figure 5 represent the microscopic observation on MCF-7 cells after treated with IC_{50} dose of *E. foetidum* leaf extracts for 24, 48 and 72 hours. In examining the untreated cells, no significant morphological changes were observed over the observation period. The cells maintained their characteristic healthy appearance, adhering to the culture flask in a uniform monolayer. According to previous studies, MCF-7 cells typically exhibit a well-defined epithelial-like morphology with uniform cellular arrangement (Dutta *et al*., 2017). However, the density of the cells was increased within 72 hours as expected due to the healthy cells still active to proliferate. Etoposide treatment shows morphology and density changes on MCF-7 cells as early as 24 hours with sign of cell rounding. As the duration of treatment increase, the cell density was reduced, and apoptosis bodies appear because of late apoptosis stage after 72 hours. For the effect of EtOH:40°C, EtOH:60°C, MeOH:40°C and MeOH:60°C extract, significant morphological changes on MCF-7 cells were observed after 48 and 72 hours. A significant reduction in cell density was observed after 24, 48 and 72 hours, indicating of inhibited cell growth. For morphological changes, round cells were identified, and the cells start to detach from the culture flask surface after 48 hours. After 72 hours, more round cells were observed. Rounding cell morphology in MCF-7 cells often indicates changes associated with cellular stress, early apoptosis, or alterations in cell adhesion (Pirsko *et al*., 2018). At IC_{50} dose, the extract was expected to inhibit 50 % of the cell population.

Variations in bioactivity based on extraction solvents and drying methods highlight the importance of careful harvesting, drying, and post-harvest practices. Harvesting at the optimal maturity stage and employing gentle drying methods are crucial for preserving heat-sensitive phytochemicals responsible for antioxidant and antiproliferative properties. This aligns with findings in other medicinal plants, where lower-temperature drying (compared to high-temperature methods) retained greater phenolic content and antioxidant activity (Snoussi *et al*., 2021; Stephenus *et al*., 2023; Babaei Rad *et al*., 2025). Furthermore, studies on *E. foetidum* essential oils have revealed that certain volatile compounds are heat-labile, undergoing degradation during high-temperature processing (Thomas *et al*., 2017).

Conclusions and Recommendations

In conclusion, *E. foetidum* leaf extracts show significant antioxidant, antimicrobial and antiproliferative activities indicating their potential for addressing oxidative stress, antimicrobial treatments and developing anticancer. However, the effectiveness of each extract in exhibit these biological activities is different between each other due to it has different combination of extraction solvent and drying temperature. The results from DPPH, ABTS and FRAP assay shows that MeOH:40°C extract of *E. foetidum* leaf demonstrated the highest antioxidant activity, followed by EtOH:40°C extract. Lower drying temperature consistently resulted in higher antioxidant activity, particularly with MeOH extracts. In the case of antimicrobial assessment by using disc diffusion method, all *E. foetidum* extracts displayed significant antimicrobial activity against *S. aureus*, with EtOH:40°C and MeOH:40°C extracts showing higher efficacy at certain concentrations. No notable antimicrobial activity was observed against *E. coli* for any extract. For antiproliferative activity, results of MTT assay shows that EtOH:40°C extract of *E. foetidum* leaf exhibited the highest antiproliferative activity against MCF-7 cells, with no significant difference from MeOH:40°C extract. Lower drying temperature at 40°C proved more effective in inhibiting MCF-7 cell proliferation. This study advocates for further research into *E. foetidum* as a valuable source of bioactive compounds, paving the way for sustainable, plant-based pharmaceuticals. It also underscores the critical importance of biodiversity conservation and preserving traditional knowledge. Future investigations should prioritize in vivo validation, safety assessments, and sustainable cultivation approaches

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Novelty Statement

This study is the first to compare the combined effects of drying temperature and solvent type on

the antioxidant, antimicrobial, and antiproliferative activities of Malaysian-grown *Eryngium foetidum*, providing new insights for optimizing its therapeutic potential.

Author`s Contribution

Syafiq Hamizan Othman: Experiment conduction, data curation and analysis, manuscript writing
Nur Azra Aliah Roslan: Experiment conduction and data analysis
Siti Aishah Abu Bakar: Supervision, project design, and manuscript editing
Dhiya Dalila Zawawi: Providing supervision on antioxidant activity
Noor Muzamil Mohamad: Providing supervision on antimicrobial activity
Tajul Afif Abdullah: Providing supervision on plant material

Generative AI or AI assisted technology statement
The authors declare that no generative AI was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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