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In vitro antimicrobial examination and efficacy of *Eryngium foetidum* L. extract for skin ointment in animal infectious dermatitis treatment

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ABSTRACT: The development of new antimicrobial formulations for the treatment of animal infectious dermatitis is important as current antimicrobial treatments can have undesirable effects. The purpose of this study was to examine the *in vitro* antimicrobial activity of an *Eryngium foetidum* L. ethanolic extract against a bacterium, *Staphylococcus pseudintermedius*; a yeast, *Malassezia pachydermatis*; and three dermatophytes, *Microsporum canis*, *Microsporum gypseum*, and *Trichophyton mentagrophytes*; all of which are the most common animal skin pathogens causing dermatitis. A viscous semi-solid crude extract was obtained at a yield of 30.6% dry weight. Quantitative analysis of the chemical constituents of the extract by spectrophotometry revealed 3.62 mg GAE/g extract of total phenolic, 1.77 mg CE/g extract of total flavonoid, and 10.11 mg/g extract of total carotenoid contents. HPLC-DAD analysis of the carotenoid content showed that β-carotene and lutein were present at 5.17 and 4.43 mg/g extract, respectively. Antimicrobial activity by broth microdilution test found that the *E. foetidum* extract had MIC, MBC and MFC values in the range of 6.25 to 25.0 mg/ml; and the time-kill test indicated that this effect was both dose and time dependent. Ointment formulations containing the extract at concentrations of 5, 7.5 and 10% w/w had high microbicidal activity against the tested pathogens and retained their activity and physical properties after repeated freeze-thaw cycles. The results of this study show the potential of *E. foetidum* extract to be developed as a novel antimicrobial formulation for treatment of infectious dermatitis in pets.

KEYWORDS: animal dermatitis, antimicrobial activity, *Eryngium foetidum* L., topical formulation

INTRODUCTION

Superficial dermatitis arising from skin infections is the most common dermatological problem in animals. In dogs and cats, this disease is generally caused by the bacterium *S. pseudintermedius*, the yeast *M. pachydermatis*, and dermatophytes such as *M. canis*, *M. gypseum*, and *T. mentagrophytes* [1]. However, these pathogens are becoming increasingly resistant to the antimicrobial agents routinely used in clinical practice such as oxacillin (methicillin resistance), ampicillin, chloramphenicol, doxycycline, enrofloxacin, gentamicin, and penicillin G for *S. pseudintermedius* [2–4]; and itraconazole, ketoconazole, and miconazole for *M. pachydermatis* and dermatophytes [5–7]. In addition, prolonged use of these drugs can cause allergic reactions, hepatotoxicity, and nephrotoxicity in some animals [8]. Therefore, there is a growing interest in developing new antimicrobial substances from natural substances that do not have these adverse effects.

Phytochemicals from some plants have shown outstanding antimicrobial and antioxidant properties that may potentially be useful for the treatment of infectious skin diseases in animals. *E. foetidum* is a medicinal herb and food native to tropical America and the Caribbean islands presently cultivated worldwide. The leaves contain many substances including flavonoids, carotenoids, terpenes, and triterpenoids such as E-2-dodecenal, dodecanoic acid, E-2-tridecenal, duraldehyde, tetradecanal, capric acid, caryophyllene oxide, and limonene [9, 10]. Several previous studies have demonstrated that *E. foetidum* has a wide spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Escherichia coli*) and the yeast *Candida albicans* [11–13]. However, the antimicrobial activity of *E. foetidum* against animal skin pathogens has not been investigated. Therefore, this study performed a phytochemical analysis of potential antimicrobial substances in *E. foetidum* extract (total phenolics, total flavonoids, and total carotenoids) and investigated the antimicrobial effect of the extract and topical formulations containing the extract on *S. pseudintermedius*, *M. pachydermatis*, *M. canis*, *M. gypseum*, and *T. mentagrophytes*.

MATERIALS AND METHODS

Plant samples and extraction

E. foetidum was purchased from a local market in Khon Kaen Province, Thailand; and the botanical identification was confirmed by Prof. Dr. Pranom Chantran-

othai, Faculty of Science, Khon Kaen University (KKU). Plant specimens were preserved in the KKU herbarium (voucher specimen J. Aiemsaard et al). Fresh leaves of *E. foetidum* were thoroughly washed with distilled water to remove any impurities, cut into small pieces, dried at 37 °C, and finely ground to powder. A Soxhlet apparatus was used in the extraction using 95% v/v ethyl alcohol as the solvent. The extraction was carried out at 80–85 °C for 24 h. The ethanolic extract was then filtered through Whatman filter paper No. 1, and the solvent was evaporated with a rotary evaporator (Heidolph, Germany) at 55 °C.

Total phenolic content

The total phenolic content of *E. foetidum* extract was determined by the Folin-Ciocalteu method. Briefly, 0.5 g of extract was dissolved in 10 ml of 98% v/v ethyl alcohol (Merck, Germany); then, 0.5 ml of the solution was mixed with 0.1 ml Folin-Ciocalteu reagent and allowed to stand for 5 min before adding 0.1 ml of 20% w/v sodium carbonate and adjusting the final volume to 1 ml with 98% ethyl alcohol. The mixture was incubated at room temperature in the dark for 90 min. The absorbance was measured at 746 nm using a UV-visible spectrophotometer (UV-160A, Shimadzu, Japan). The total phenolic content was calculated using a standard calibration curve of gallic acid (Sigma-Aldrich, Germany), and the results are expressed as gallic acid equivalent in mg/g of crude extract (mg GAE/g extract) [14].

Total flavonoid content

The total flavonoid content was determined by aluminum chloride assay. In brief, 0.5 g of the extract was mixed with 0.05 ml of 0.5% w/v sodium nitrite and left for 5 min; 0.05 ml of 10% w/v aluminum chloride was added and allowed to stand for 5 min; 0.5 ml of 1 M sodium hydroxide was added; and, then, the final volume was adjusted to 1.5 ml with 98% v/v ethyl alcohol. The absorbance was measured at 510 nm. The total flavonoid content was calculated using a standard calibration curve of catechin (Sigma-Aldrich), and the results are expressed as catechin equivalent in mg/g of crude extract (mg CE/g extract) [14].

Carotenoid content

The extraction of carotenoids and the determination of their total content were performed according to a method described by Sukto et al [15] with modifications. Briefly, the *E. foetidum* extract was dissolved with dimethyl sulfoxide (DMSO; Sigma-Aldrich) to give a final concentration of 100 mg/ml. The carotenoids were then extracted by mixing 1 ml of the sample with ethyl alcohol to obtain a final volume of 50 ml. Ten milliliters of diethyl ether were added, and the mixture was washed 3 times with deionized water. The diethyl ether fraction was separated and added to 10 ml of 10% w/v ethanolic potassium hydroxide and left overnight. The mixture was then washed 3 times with deionized water to obtain the carotenoid fraction. The absorbance of the carotenoid fraction was measured at 450 nm, and the total carotenoid content was calculated using the following formula:

$$C = A/(\varepsilon \times I)$$

where, C is the total carotenoid concentration of carotenoid extract (g/ml), A is the absorbance at 450 nm, I is the length of the light path (1 cm), and ε is the absorption coefficient defined as $A^{1\%}_{1\ \mathrm{cm}}$ of beta-carotene in ethanol (2,592).

The carotenoid content was analyzed by high performance liquid chromatography with diode-array detection (HPLC-DAD) (Shimadzu, Japan) using a YMC C30 column (5 μm, 4.6×250 mm) as the stationary phase. The mobile phases were methyl alcohol (A), methyl tertiary-butyl ether (B), and deionized water (C). The injection volume was 20 μl, and the total analysis time was 20 min at 25 °C with detection at a wavelength of 450 nm. The analysis conditions were as follows: A:B:C at 0–2 min was 78.4:20:1.6; at 2–2.01 min was 58.8:40:1.2 (rate of 1.4 ml/min); at 2.01–12 min was 57:40:3; at 12–13 min was 0:100:0; and at 13–20 min was 78.4:20:1.6 (rate of 1.0 ml/min). The retention times and peak areas of the analyzed samples were calculated using a standard calibration curve of β-carotene and lutein (Sigma-Aldrich) [16].

Broth microdilution method

S. pseudintermedius ATCC 49051 and *M. pachydermatis* ATCC 14522 were purchased from the American Type Culture Collection (ATCC), Virginia, USA. The dermatophytes, *M. canis* DMST 29297, *M. gypseum* DMST 21146, and *T. mentagrophytes* DMST 19735, were obtained from Department of Medical Sciences, Nonthaburi, Thailand. Broth microdilution testing was performed according to CLSI guidelines [17, 18] with some modifications. Briefly, the *E. foetidum* extract was dissolved in DMSO and serially two-fold diluted (50 μl) with Mueller-Hinton broth (MHB) for bacteria, or Sabouraud dextrose broth (SDB) for yeast and molds (both MHB and SDB were from Becton Dickinson, France) in 96-well round-bottomed microtiter plates (Corning Incorporated, USA). Fifty microliters of the appropriate microbial suspensions (1×10^6 CFU/ml of *S. pseudintermedius* and 2×10^3 CFU/ml of yeast and molds) were separately added to wells. The bacteria and the yeast plates were incubated at 37 °C for 24 h, while the mold plates were incubated at 30 °C for 72 h. Wells containing broth with and without microbial suspension were positive and negative growth controls, respectively. The minimum inhibitory concentration (MIC) was determined from the lowest concentration of the extract inhibiting visible growth

after incubation. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined from the lowest concentration of the extract that inhibited growth on Mueller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for fungi (both MHB and SDB were from Becton Dickinson). Cephalexin, gentamicin, and ketoconazole were used as standard antimicrobial controls (all drugs were from Sigma-Aldrich).

Time-kill test

The time-kill assay was performed according to the method previously described by Aiemsaard et al [19] with some modifications. Briefly, 100 µl of microbial inocula containing 1×10^7 CFU/ml of *S. pseudintermedius* and 1×10^4 CFU/ml of yeast and molds were mixed with 900 µl of diluted *E. foetidum* extract (in normal saline solution) to give a final concentration of 1-, 5-, and 10-times MIC. After incubation at 30 °C for 15 and 30 min, 1, 3, 6, and 24 h, the mixture was 10-fold diluted with normal saline solution, and 10^{-1} to 10^{-3} dilutions were inoculated onto MHA or SDA plates. After incubation at 37 °C for 24 h (bacterium and yeast) or 30 °C for 96 h (molds), the number of recovered colonies was recorded, and the results were expressed as $\log_{10}$ survival and $\log_{10}$ reduction of viable cells (CFU/ml). Normal saline solution and DMSO (62.5 µl/ml) were used as negative control and diluent control, respectively.

Formulation preparation

The *E. foetidum* extract was incorporated into three topical ointment formulations (Table 1) [20]. Polyethylene glycol 4000 was mixed with polyethylene glycol 400, melted on a water bath and allowed to cool to 60 °C (A-phase). The *E. foetidum* extract, methylparaben, propylparaben, and distilled water were dissolved in propylene glycol and heated to 60 °C (B-phase). The B-phase was thoroughly mixed with the A-phase. The mixture was removed from the heat and stirred until it began to congeal. All compositions were pharmaceutical grade and obtained from Union Science Trading Co., Ltd. (Khon Kaen, Thailand).

Antimicrobial activity of *E. foetidum* ointment formulations

The antimicrobial efficacy of the *E. foetidum* ointment formulations was investigated by time-kill test. Briefly, 100 µl of microbial suspension (1×10^7 CFU/ml of *S. pseudintermedius* and 1×10^4 CFU/ml of yeast and molds) was separately mixed with 900 mg (approximately 900 µl) of each ointment formulation. After incubation at 30 °C for 15 and 30 min, 1, 3, 6, and 24 h, the mixture was 10-fold diluted with normal saline solution; and 10^{-1} to 10^{-3} dilutions were inoculated onto MHA or SDA plates. After incubation at 37 °C for 24 h (bacterium and yeast) or 30 °C for 96 h (molds), the number of recovered colonies was recorded; and the results were expressed as $\log_{10}$ survival and $\log_{10}$ reduction of viable cells (CFU/ml) [19].

Stability testing of *E. foetidum* ointment formulations

The time-kill kinetics and physical properties of the developed formulations were assessed before and after six freeze-thawing cycles of 24 h at −5 °C followed by 24 h at 40 °C [21]. The physical properties of the formulations that were measured were pH (Lab 850 set pH meter, SI Analytics, Germany) and viscosity (Brookfield DVE viscometer, Ametek Brookfield, USA); and the color, presence of sediment and any fractionation in the formulations was assessed by visual observation.

Statistical analysis

Each experiment was performed in triplicate. The normality of the data was assessed by the Shapiro-Wilk test. Differences in $\log_{10}$ viable cell reduction, pH value, and viscosity were analyzed by using the paired sample t-test (for data with a normal distribution) and the Wilcoxon signed-rank test (for data without a normal distribution). The analysis was performed on IBM SPSS v.28 software using $p \leqslant 0.05$ as significant.

RESULTS

Phytochemical content

The total phenolic, total flavonoid, and carotenoid contents of *E. foetidum* extract were shown in Table 2. The major component of *E. foetidum* ethanolic extract was carotenoids (10.11 mg/g extract) consisting of 5.17 mg/g extract β-carotene and 4.43 mg/g extract lutein. Total phenolic (3.62 mg GAE/g extract) and total flavonoid (1.77 mg CE/g extract) contents were 2.8 and 5.7-times lower than the total carotenoid content, respectively.

Antimicrobial activity of *E. foetidum* extract

The broth microdilution testing demonstrated that *E. foetidum* extract MICs were relatively higher than the MICs for the respective antimicrobial controls; cephalexin, gentamicin, and ketoconazole. For *S. pseudintermedius*, all MBC values were two times (one dilution) higher than their respective MIC values; while for the yeast and dermatophytes, the MFC values were the same as their respective MICs (Table 3). For *E. foetidum* extract, MIC, MBC, and MFC values were in the range of 6.25–25.00 mg/ml against all tested microorganisms. Cephalexin and gentamicin showed MICs against *S. pseudintermedius* of 0.0078 and 0.0010 µg/ml, respectively. The MIC and MFC values of ketoconazole against *M. pachydermatis*, *M. canis*, *M. gypseum*, and *T. mentagrophytes* ranged from 0.0003 to 0.0078 µg/ml. *T. mentagrophytes* showed the most resistance to killing by *E. foetidum* extract, with an MIC

Table 1 Ingredients of *E. foetidum* ointment formulations.

Ingredient	Amount (g)		
	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)
E. foetidum extract	5.00	7.50	10.00
Methylparaben	0.12	0.12	0.12
Propylparaben	0.08	0.08	0.08
Propylene glycol	5.00	5.00	5.00
Polyethylene glycol 400	59.40	56.90	54.40
Polyethylene glycol 4000	25.00	25.00	25.00
Distilled water	0.40	0.40	0.40

Table 2 Chemical composition of *E. foetidum* extract.

Constituent	Amount (per g extract)
Total phenolic	3.62 mg GAE
Total flavonoid	1.77 mg CE
Total carotenoid	10.11 mg
β-carotene	5.17 mg
Lutein	4.43 mg

two times that of *S. pseudintermedius* and *M. pachydermatis*, and four times that of *M. canis* and *M. gypseum*.

Time-kill kinetics of *E. foetidum* extract

Fig. 1 shows the time-kill kinetics of ethanolic *E. foetidum* extract against *S. pseudintermedius*, *M. pachydermatis*, *M. canis*, *M. gypseum*, and *T. mentagrophytes*. The *E. foetidum* extract showed a bactericidal or fungicidal effect (greater than 3-$\log_{10}$ reduction in the number of viable cells) against the tested microbial strains at concentrations ranging from 62.5 to 250 mg/ml. For *S. pseudintermedius*, *E. foetidum* extract was bactericidal at concentrations of 62.5 mg/ml (5×MIC) at 6 h and 125 mg/ml (10×MIC) at 15 min. For *M. pachydermatis*, *E. foetidum* extract was fungicidal at concentrations of 62.5 mg/ml (5×MIC) at 3 h and 125 mg/ml (10×MIC) at 15 min. For *M. canis* and *M. gypseum*, *E. foetidum* extract was fungicidal at a concentration of 62.5 mg/ml (10×MIC) at 3 h and 1 h, respectively. Finally, *E. foetidum* extract was fungicidal to *T. mentagrophytes* at concentrations of 125 mg/ml (5×MIC) at 6 h and 250 mg/ml (10×MIC) at 30 min. Thus, *E. foetidum* extract at 10 times MIC was lethal to all tested microbes; and at 5 times MIC, it was lethal to *S. pseudintermedius*, *M. pachydermatis*, and *T. mentagrophytes*, indicating that the antimicrobial effect of the *E. foetidum* extract was dose and concentration dependent.

Physical properties of *E. foetidum* ointment

Three ointment formulations containing 5%, 7.5%, and 10% w/w *E. foetidum* extract were prepared. The ointments were all semisolid with a shiny appearance and dark-green in color. The three ointment formulations had similar pH values (5.52±0.34 to 5.65±0.10) and high viscosities although the ointment containing 5% w/w extract had a lower viscosity (45,233±929 cP) than the 7.5% and 10% w/w extract ointments (92,833±1,716 and 80,267±1,250 cP, respectively). After freeze-thawing, the pH and viscosity of each formulation did not significantly change (all p-value > 0.05; Table 4). Fractionation, separation and sedimentation of ointment components were not observed in any formulations over the time course of the study.

Antimicrobial activity of *E. foetidum* ointment formulations

The time-kill kinetics of the developed ointment formulations both before and after repeat freeze-thaw cycles were shown in Fig. 2. All formulations successfully eradicated each of the tested pathogens. The 5%, 7.5%, and 10% w/w extract ointment formulations were bactericidal/fungicidal to *S. pseudintermedius* and *M. pachydermatis* at 3 h, while the freeze-thawed 5% w/w extract ointment formulation was only bactericidal/fungicidal to these strains at 6 h. For *M. canis*, *M. gypseum*, and *T. mentagrophytes*, all three ointment formulations were fungicidal at 15 min regardless of freeze-thaw treatment.

DISCUSSION

The phytochemical analyses performed in this study confirmed that the ethanolic extract of *E. foetidum* contained phenolic, flavonoid, and carotenoid compounds. A previous study reported that *E. foetidum* extract contained polyphenols at 2.18–2.58 mg/g dry plant, flavonoids at 1.14–1.80 mg/g dry plant, and carotenoids at 2.89–5.15 mg/g dry plant [22]. Extraction of *E. foetidum* with different solvents can affect the concentration of these key constituents. Kaewnarin et al [14] found that ethanolic extract had total phenolic and total flavonoid contents of 0.07 mg GAE/g extract and 0.009 mg quercetin equivalent (QE)/g extract, respectively; while ethyl acetate extract contained 0.05 mg GAE/g extract and 0.019 mg QE/g extract, respectively. A study by Swargiary et al [23] showed that methanolic extract of *E. foetidum* contained total phenolics of 38.10 mg GAE/g extract and total flavonoids of

Table 3 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of *E. foetidum* extract.

Microorganism	*E. foetidum* extract (mg/ml)		Cephalexin (μg/ml)		Gentamicin (μg/ml)		Ketoconazole (μg/ml)		DMSO (μl/ml)	
	MIC	MBC/MFC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MBC/MFC
S. pseudintermedius	12.50	25.00	0.0078	0.0156	0.0010	0.0020	–	–	>125	>125
M. pachydermatis	12.50	12.50	–	–	–	–	0.0078	0.0078	>125	>125
M. canis	6.25	6.25	–	–	–	–	0.0003	0.0003	>125	>125
M. gypseum	6.25	6.25	–	–	–	–	0.0020	0.0020	>125	>125
T. mentagrophytes	25.00	25.00	–	–	–	–	0.0078	0.0078	>125	>125

– = not tested.

Table 4 Viscosity and pH (mean ± SD) of *E. foetidum* ointment formulations before and after freeze-thaw (FT) cycles (measured at 25 °C).

Formulation	pH		Viscosity (cP)	
	Before FT	After FT	Before FT	After FT
5% w/w (F1)	5.52 ± 0.34	4.80 ± 0.01	45,233 ± 929	38,833 ± 4,311
p-value	0.069 *		0.161 *	
7.5% w/w (F2)	5.55 ± 0.01	5.31 ± 0.01	92,833 ± 1,716	85,247 ± 4,004
p-value	0.083 **		0.137 *	
10% w/w (F3)	5.65 ± 0.10	5.47 ± 0.01	80,267 ± 1,250	73,367 ± 1,850
p-value	0.102 **		0.059 *	

* Paired sample *t*-test. ** Wilcoxon signed-rank test.

13.69 mg QE/g extract. Kaushal [24] found that *E. foetidum* aqueous extract had a total phenolic concentration of 3.11±0.02 mg GAE/g extract.

The antimicrobial testing indicated that *E. foetidum* extract was active against the main groups of pathogens causing animal dermatitis including *S. pseudintermedius*, *M. pachydermatis*, *M. canis*, *M. gypseum*, and *T. mentagrophytes*, although a high concentration (62.5–125 mg/ml) was required. Interestingly, when the *E. foetidum* extract was prepared in ointment formulations, it was found that the formulations showed improved antimicrobial effects. Strikingly, the three formulations, 5% w/w (50 mg/g), 7.5% w/w (75 mg/g), and 10% w/w (100 mg/g), eradicated the tested dermatophytes within 15 min, even after repeated freeze-thaw cycles. While the antimicrobial activity of the formulations was less profound against the bacterium *S. pseudintermedius* and the yeast *M. pachydermatis*, the ointments were still able to eradicate these microbes within 3 h. Several previous studies have shown broad-spectrum activity of *E. foetidum* extracts against pathogenic microorganisms including the bacteria *S. aureus*, *P. aeruginosa*, *E. coli*, *B. cereus*, and *Vibrio cholerae* (MIC range of 0.003–2.5 mg/ml) and the yeast *C. albicans*, *Candida guilliermondi* and *Cryptococcus neoformans* (MIC range of 0.002–2.5 mg/ml) [25–27]. However, this is the first study to show antimicrobial activity of *E. foetidum* extracts against pathogenic dermatophytes.

Phytochemicals are secondary metabolites produced by plants that have long been known for their medicinal benefits in both traditional and modern medicines. Phenolic compounds are a large group of phytochemicals with hundreds of substances and their derivatives including flavonoids [28]. The phenolic hydroxyl group shows high protein binding affinity and lipophilicity, and phenolic compounds have been shown to bind to microbial cell membranes, disrupting the membrane structure and the functions of substances and organelles within cells [29, 30]. Carotenoids are yellow, orange, red, and purple pigments that give different colors to plants and are also found in many bacteria, fungi, and algae. Although these compounds are not predominantly antimicrobial, they show strong antioxidant properties that can be effective in the treatment of dermatitis [31]. Antioxidants act to decrease oxidative stress by reducing the amount of reactive oxygen species and, hence, reduce or prevent the inflammatory processes that increase the severity of lesions in dermatitis [32].

Ointment formulations are topical drugs that have very good skin adhesion properties and also act to hydrate the skin by protecting it from water loss. Good adhesion to the animal's skin allows the drug to remain active and last for a long time in the lesion site [33, 34]. The lipophilic properties of ointment bases make them compatible with lipophilic active plant compounds and

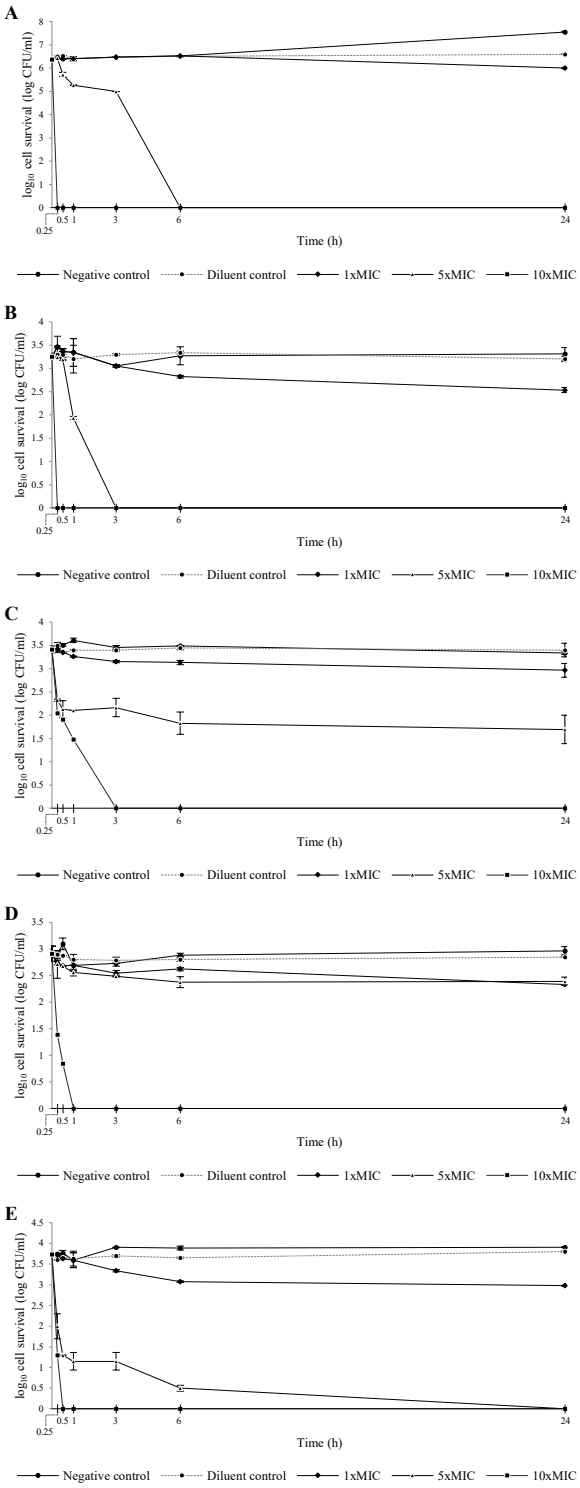

Fig. 1 Time-kill kinetics of *E. foetidum* extract against *S. pseudintermedius* ATCC 49051 MIC = 12.50 mg/ml (A), *M. pachydermatis* ATCC 14522 MIC = 12.50 mg/ml (B), *M. canis* DMST 29297 MIC = 6.25 mg/ml (C), *M. gypseum* DMST 21146 MIC = 6.25 mg/ml (D), and *T. mentagrophytes* DMST 19735 MIC = 25.00 mg/ml (E). Negative control = normal saline solution. Diluent control = DMSO in normal saline solution (62.5 µl/ml).

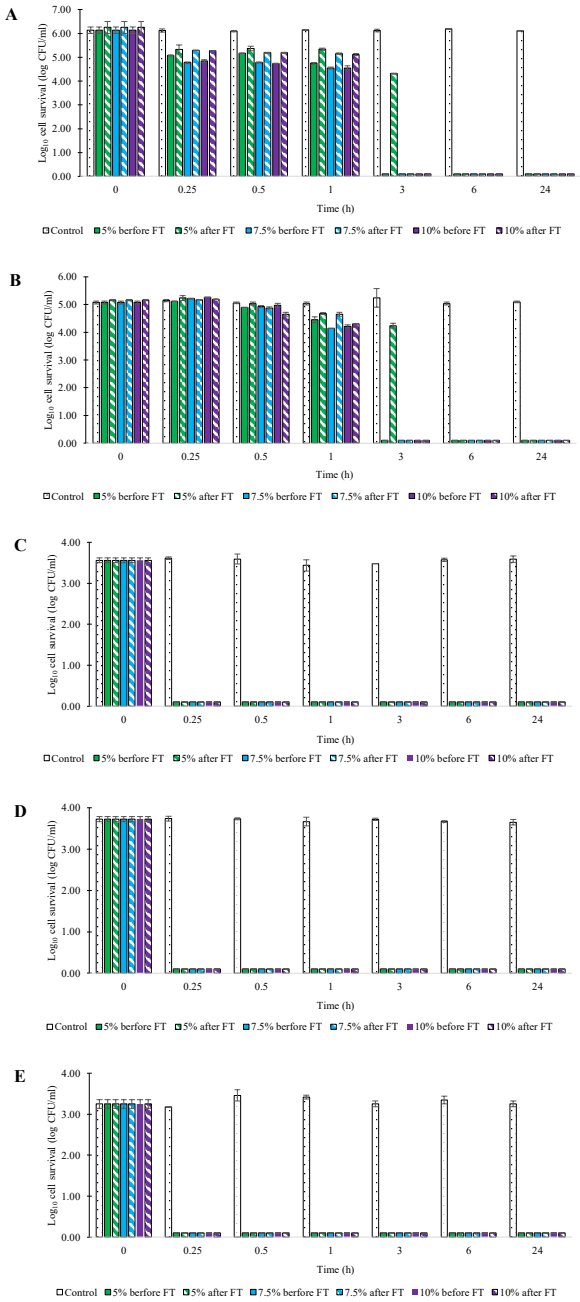

Fig. 2 Time-kill kinetics of 5, 7.5, and 10% w/w *E. foetidum* extract ointment formulations against *S. pseudintermedius* ATCC 49051 (A), *M. pachydermatis* ATCC 14522 (B), *M. canis* DMST 29297 (C), *M. gypseum* DMST 21146 (D), and *T. mentagrophytes* DMST 19735 (E) before and after freeze-thawing (FT). Control = normal saline solution.

promote their antimicrobial and antioxidant activities. The materials used as the ointment base in the current study were the water-soluble PEG 400 and 4000 polymers. They have both hydrophilic and hydrophobic properties and are biocompatible with body tissues not

causing skin irritation or toxicity. Additionally, they have been shown to promote the regeneration of skin tissue [35].

In conclusion, *E. foetidum* extract has the potential to be developed as an antimicrobial ointment with activity against *S. pseudintermedius*, *M. pachydermatis*, *M. canis*, *M. gypseum*, and *T. mentagrophytes*, the most common agents of infectious dermatitis in animals. Additional studies to investigate the clinical efficacy of *E. foetidum* extract and its formulations are necessary.

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