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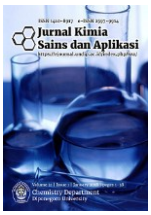

Antibacterial Edible Film Based on *Trema orientalis* Leaf Extract: In Silico, In Vitro, and Toxicity Screening

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Abstract

Antibacterial edible films based on natural extracts have gained attention as sustainable alternatives for food packaging. Leaf extract of *Trema orientalis* contains bioactive compounds, including flavonoids, phenolics, alkaloids, terpenoids, and saponins, with potential antibacterial activity. The extract was obtained using ethanol and characterized by LC–MS, which identified 16 compounds, with an extraction yield of 16.45%. Edible films were prepared using a polyvinyl alcohol (PVA)–polyethylene glycol (PEG) matrix and characterized by FTIR, SEM, and physical–mechanical analyses. The incorporation of the extract improved the film properties, including thickness, water resistance, and tensile strength. Antibacterial activity against *S. aureus* and *E. coli* increased with extract concentration, reaching a maximum inhibition zone of 13.3 ± 0.58 mm at 60% extract concentration. Molecular docking against DNA gyrase B of *E. coli* (6F86) and *S. aureus* (4URM) identified compound 5 (-8.04 kcal/mol) and compound 13 (-7.94 kcal/mol) as the strongest-binding compounds toward their respective target proteins, indicating their potential contribution to the antibacterial activity of the extract. Toxicity evaluation using the Brine Shrimp Lethality Test (BSLT) yielded an LC_{50} value of 1976 ppm, indicating that the extract was non-toxic. These findings demonstrate that *Trema orientalis* extract-based edible films exhibit promising antibacterial properties and have potential as environmentally friendly active food packaging materials.

1. Introduction

The development of biopolymer-based film materials has increased significantly in recent years, particularly in the form of edible films. Edible films are thin layers composed of natural polymers and can be formulated with various functional compounds [1]. This characteristic makes edible films a flexible system for studying polymer matrices and the active compounds dispersed within them, particularly from the perspective of chemistry and materials science [2].

One of the major directions in edible film development is the incorporation of antibacterial agents to produce materials with additional biological functionality. Natural-based antibacterial agents are considered promising due to their diverse chemical structures and their relatively high compatibility with biopolymer matrices [3]. In addition, natural compounds generally contain various reactive functional groups that enable interactions with target microorganisms through multiple mechanisms, thereby potentially providing more complex biological activities compared to single synthetic compounds [4].

Plant extracts are rich in various phytochemicals, such as phenolics, flavonoids, and alkaloids, which have been widely reported to exhibit antibacterial activity. These biological activities are closely associated with their molecular structures, including the presence of hydroxyl groups, aromatic systems, and charge distribution that contribute to interactions with microbial cell components [5]. Therefore, understanding the relationship between chemical structure and biological activity is an important aspect in the utilization of plant extracts as active agents, particularly when these

compounds are integrated into a material system such as an edible film.

Trema orientalis is one of the plant species reported to contain various bioactive compounds and has been investigated for its potential biological activities, including antibacterial activity [6]. Previous studies have shown that *T. orientalis* extracts possess antioxidant, antimicrobial, and cytotoxic properties, which are closely associated with their phytochemical constituents and demonstrate the plant's promising medicinal potential [7, 8]. However, these studies have primarily focused on evaluating the biological activities of the extracts themselves. The application of *T. orientalis* leaf extract in edible film systems remains limited, and comprehensive studies integrating edible film development with physicochemical characterization, LC-MS-based compound identification, molecular docking analysis, antibacterial evaluation, and preliminary toxicity assessment are still scarce. Therefore, the present study aimed to develop an antibacterial edible film based on *T. orientalis* leaf extract and to comprehensively evaluate its physicochemical properties, antibacterial activity, toxicity, and the molecular interactions of the identified compounds using LC–MS and molecular docking.

In silico approaches, such as molecular docking, can be utilized to predict the interactions between bioactive compounds and microbial target proteins, thereby providing preliminary insights into the mechanisms of action of active compounds and assisting in the interpretation of experimental findings [9]. Nevertheless, in vitro assays are still required to confirm the antimicrobial activity of edible films containing plant extracts under controlled experimental conditions.

In addition to biological activity testing, toxicity screening is an important part of the preliminary evaluation of bioactive compound-based materials, particularly in systems involving potential direct contact [10]. This evaluation is not intended to provide a comprehensive safety claim, but rather to serve as a preliminary screening to obtain a general overview of potential toxic effects, thereby supporting the more responsible development of the material.

Based on the aforementioned background, this study aims to investigate antibacterial edible films based on *T. orientalis* leaf extract (TOEE) through an integrated approach involving in silico, in vitro, and toxicity screening analyses. Molecular docking was employed to predict the interactions between the identified bioactive compounds and bacterial target proteins, while in vitro antibacterial assays were performed to experimentally evaluate the antibacterial activity of the edible films. In addition, toxicity screening was conducted to provide a preliminary assessment of the safety of the developed material. This integrated approach is expected to provide a more comprehensive understanding of the antibacterial potential, possible molecular mechanisms, and preliminary safety profile of *T. orientalis*-based edible films.

2. Experimental

2.1. Instrument and Materials

The instruments used in this study included a distillation apparatus and a rotary evaporator (Buchi, Switzerland). Laminar Airflow cabinet, oven (B-One, Indonesia), analytical balance (Memmert, Germany), Hotplate (DLAB, China), and a set of glassware. The materials used were technical-grade ethanol, nutrient agar (Merck, Germany), paper discs, dimethyl sulfoxide (DMSO) (Merck, Germany), chloramphenicol, polyvinyl alcohol (PVA), polyethylene glycol 400 (PEG-400), and distilled water.

2.2. Sample Preparation

T. orientalis leaves were washed and air-dried at room temperature. The dried leaves were then blended to obtain simplicia powder. The simplicia powder was macerated in ethanol at a 1:10 ratio in a dark bottle for 3 × 24 h. The mixture was filtered using filter paper to obtain the filtrate. The resulting filtrate was subsequently concentrated using a rotary evaporator to obtain a viscous extract.

2.3. Phytochemical Screening

Phytochemical screening of the concentrated TOEE was performed using phytochemical reagents to detect secondary metabolites, including flavonoids, alkaloids, saponins, tannins, terpenoids/steroids, and phenolic compounds.

2.4. Liquid Chromatography Mass Spectrometry (LC- MS) Characterization

A total of 1 g of the concentrated TOEE was analyzed using an LC-MS instrument. The analysis was conducted in 23 min, and the spectral data were processed using SIRIUS version 5.8.6 and Masslynx to assist with compound identification based on mass fragmentation patterns.

2.5. Molecular Docking

The three-dimensional crystal structures of *E. coli* DNA gyrase B (PDB ID: 6F86) and *S. aureus* DNA gyrase B (PDB ID: 4URM) were retrieved from the RCSB Protein Data Bank. The native ligands were separated from the protein structures using UCSF Chimera and re-docked into their respective binding sites using AutoDock Vina [11]. The docking protocol was validated by calculating the Root Mean Square Deviation (RMSD) between the crystallographic and re-docked ligand conformations using PyMOL. An RMSD value of less than 2.0 Å was considered indicative of a valid docking protocol [12]. Subsequently, all compounds identified from the revised LC-MS analysis were docked into the validated binding sites. Chloramphenicol was included as a positive control for comparison, and the binding affinity values (kcal/mol) were used for further analysis.

Table 1. Edible film formulation

Composition	Formulation				
	EF10%	EF20%	EF30%	EF40%	EF50%
PVA (g)	1	1	1	1	1
PEG-400 (mL)	0.25	0.25	0.25	0.25	025
100% stock extract solution (mL)	2.5	5.0	7.5	10.0	15.0
Distilled water (mL)	q.s to 25	q.s to 25	q.s to 25	q.s to 25	q.s to 25

2.6. Formulation of Edible Film

The edible films were prepared using PVA as the film-forming polymer and PEG-400 as the plasticizer. A 100% stock extract solution of TOEE was prepared prior to film formulation. Different volumes of the stock extract solution were incorporated to obtain final extract concentrations of 10%, 20%, 30%, 40%, and 60% (v/v) in the film-forming solution. Distilled water was used as the solvent and was added to adjust the final volume of each formulation to 25 mL. The formulation details are presented in Table 1.

PVA was dissolved in distilled water under continuous stirring and heating until a homogeneous solution was obtained. PEG-400 was then added as a plasticizer, followed by the addition of the required volume of the 100% stock extract solution according to the formulation. Distilled water was subsequently added to each formulation to adjust the final volume to 25 mL before casting. The film-forming solution was cast into Petri dishes and dried in an oven at 60°C for 5 h until edible film sheets were formed.

2.7. Thickness

The thickness of the edible films was measured using a thickness gauge with an accuracy of 0.01 mm. Measurements were taken at five different points, and the thickness value was calculated as the average of the obtained measurements (Equation 1).

$$Thickness\ (mm) = \frac{p_1+p_2+p_3+p_4+p_5}{5} \quad (1)$$

2.8. Water Absorption

The water absorption test was conducted according to the method described by Khotimah *et al.* [13]. The edible films were cut into 1 × 5 cm pieces and weighed to determine the initial weight (W_1). The samples were then immersed in distilled water for 1 min. After immersion, excess water on the film surface was gently removed using tissue paper, and the films were weighed again to obtain the final weight (W_2). Water absorption was calculated using Equation 2.

$$Water\ absorption\ (\%) = \frac{W_1-W_2}{W_1} \times 100 \quad (2)$$

Where W_1 is the initial weight of the sample (g), and W_2 is the final weight after immersion (g).

2.9. Tensile Strength

The tensile strength of the edible films was measured using a tensile tester. The samples were cut into dimensions of 3 × 15 cm and clamped at both ends along the length of the film. After the test was completed, the maximum tensile force was recorded, and the tensile strength was calculated using Equation 3, according to the method described by Bochnia *et al.* [14].

$$T = \frac{F}{A} \quad (3)$$

Where T is the tensile strength (MPa), F is the maximum tensile force (N), and A is the cross-sectional area of the specimen (mm^2), calculated as the product of the film width (mm) and thickness (mm).

2.10. Antibacterial Activity

The antibacterial activity of the extracts and edible films was evaluated using the disc diffusion method. The extract concentrations used ranged from 10–80%, while the edible film concentrations ranged from 10–60%. Nutrient agar medium was prepared by dissolving the medium, sterilizing it in an autoclave, and pouring it into Petri dishes until solidified. The solidified nutrient agar medium was evenly inoculated with the bacterial suspension using the swab method. Paper discs containing extract solutions at each concentration, as well as edible film samples, were then placed on the surface of the medium. DMSO was used as the negative control, while chloramphenicol served as the positive control.

2.11. Toxicity Screening

Toxicity screening was carried out using *Artemia salina* larvae through the Brine Shrimp Lethality Test (BSLT) according to the procedure described by Istiqomah *et al.* [15]. Hatched larvae were used as test organisms and exposed to the TOEE at concentrations ranging from 0–1000 ppm with three replications. Each treatment was incubated for 24 h at room temperature without feeding. After incubation, the number of dead larvae was counted to determine the mortality percentage and LC_{50} value.

3. Results and Discussion

3.1. Extract Yield

The extract obtained from the extraction process is presented in Table 2.

Table 2. TOEE Yield

Sample	Simplicia weight (g)	Extract weight (g)	Yield (% w/w)
TOEE	500	82.29	16.45

3.2. Phytochemical Result

Based on Table 3, the phytochemical screening results showed that TOEE contains flavonoids, saponins, terpenoids/steroids, and phenolic compounds, as indicated by characteristic color changes or precipitate formation with each reagent. The alkaloid test showed a positive result with Mayer's reagent, but negative results with Wagner's and Dragendorff's reagents, suggesting the presence of alkaloids in limited amounts. Overall, the presence of these compounds may contribute to the biological activity of *T. orientalis* leaf extract.

Table 3. Phytochemical result of TOEE

Compound class	Reagent	Result
Flavonoid	HCl + Mg	(+)
Saponin	Distilled water	(+)
Terpenoid/Steroid	Lieberman Burchard	(+)
Alkaloid	Mayer's	(+)
	Wegner	(-)
	Dragendorff	(-)
Phenolic	$FeCl_3$	(+)
Tannin	$FeCl_3$ + H_2SO_4	(+)

3.3. Liquid Chromatography–Mass Spectrometry Characterization

The LC-MS chromatogram of *T. orientalis* leaf extract is presented in Figure 1, while the identified metabolites are summarized in Table 4. LC-MS analysis identified 16 compounds. The identified compounds, together with their retention times, molecular formulas, molecular weights, compound names, and chemical classes, are presented in Table 4. The identified metabolites comprised several chemical classes, including amino acids, flavonoids, flavonoid glycosides, sphingolipids, sterols, and other lipid-derived compounds.

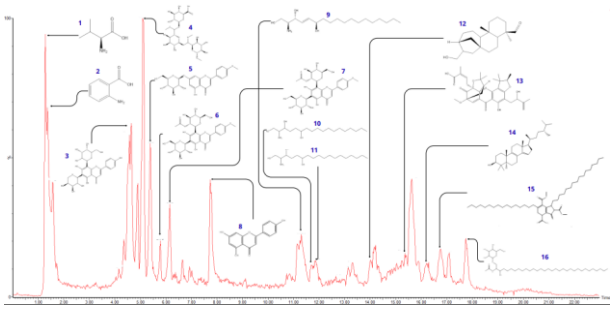

Figure 1. LC-MS chromatogram of TOEE

Table 4. LC–MS profile of compounds identified in TOEE

RT (min)	Chemical formula	Mol weight (g/mol)	Compound name	Compound class
1.28	$C_5H_{11}NO_2$	117.15	L-Valine	Amino acid
1.37	$C_7H_7NO_2$	137.14	Anthranilic Acid	Aromatic amino acid derivative
4.64	$C_{26}H_{28}O_{14}$	564.50	Isoschaftoside	Flavonoid glycoside
5.12	$C_{21}H_{36}O_{19}$	592.50	(2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[[(2R,3R,4S,5R,6S)-3,4,5-trihydroxy-6-[[(2R,3S,4S,5R,6R)-3,4,5-trihydroxy-6-methoxyoxan-2-yl]methoxymethoxy]oxan-2-yl]methoxymethoxy]oxane-2-carboxylic acid	Carbohydrate glycoside
5.39	$C_{22}H_{22}O_{10}$	446.40	Tilianin	Flavonoid glycoside
5.79	$C_{30}H_{34}O_{16}$	650.60	[(2S,3R,4S,5S,6R)-2-[5,7-dihydroxy-2-(4-methoxyphenyl)-4-oxo-6-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-8-yl]-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl]acetate	Flavonoid glycoside
6.16	$C_{30}H_{34}O_{16}$	650.60	[(2S,3R,4S,5S,6R)-2-[5,7-dihydroxy-2-(4-methoxyphenyl)-4-oxo-6-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]chromen-8-yl]-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl]acetate	Flavonoid glycoside
7.73	$C_{15}H_{10}O_5$	271.24	Apigenin	Flavonoid
11.31	$C_{18}H_{37}NO_3$	315.50	(2S,3R,6R)-2-aminooctadec-4-ene-1,3,6-triol	Sphingolipid
11.71	$C_{18}H_{39}NO_3$	317.50	6-Hydroxy dihydrosphingosine	Sphingolipid
11.85	$C_{18}H_{39}NO_3$	317.50	6-Hydroxy dihydrosphingosine	Sphingolipid
14.11	$C_{20}H_{32}O_2$	304.50	(1S,5R,9S,13R)-14-(hydroxymethyl)-5,9-dimethyltetracyclo[11.2.1.01,10.04,9]hexadecane-5-carbaldehyde	Diterpenoid
15.37	$C_{34}H_{40}O_{10}$	608.20	Scortechinone R	Prenylated flavonoid
16.20	$C_{29}H_{48}O_2$	428.70	(3S,5R,10S,13R,17R)-17-[(2S,5R)-5-hydroxy-6-methylheptan-2-yl]-4,4,10,13-tetramethyl-1,2,3,5,6,7,11,12,16,17-decahydrocyclopenta[a]phenanthren-3-ol	Phytosterol (Steroid)
16.75	$C_{46}H_{79}NO_6$	742.10	Dimethyl7-carbamoyl-3,5-dihexadecyl-1-oxo-2,3,6,7-tetrahydroindene-2,4-dicarboxylate	Lipid derivative
17.75	$C_{40}H_{79}NO_8$	702.10	*N*-[3-hydroxy-1-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxydotriacontan-2-yl]acetamide	Glycosphingolipid

3.4. Molecular Docking

The superimposition of the native and re-docked ligands for *S. aureus* (PDB ID: 4URM) and *E. coli* (PDB ID: 6F86) is presented in Figure 2. The RMSD values obtained were 0.710 Å for *S. aureus* and 1.566 Å for *E. coli*. Both values were below the acceptable threshold of 2.0 Å, indicating that the docking protocol was successfully validated and suitable for subsequent molecular docking analysis.

Following validation, all identified compounds were docked into the active sites of the target proteins using the validated docking protocol. The binding affinity values obtained for *S. aureus* and *E. coli* are presented in Table 5. The docking results showed variations in binding affinity among the tested compounds, indicating differences in their predicted binding strength toward the target proteins. In general, compounds with lower (more negative) binding affinity values were predicted to form more stable ligand–protein complexes and therefore exhibited greater potential as antibacterial agents.

As shown in Table 5, compound 5 exhibited the strongest binding affinity toward *E. coli* DNA gyrase B (6F86) with a binding affinity of -8.04 kcal/mol, followed by compounds 3 (-7.79 kcal/mol), 14 (-7.65 kcal/mol), and 13 (-7.57 kcal/mol). In contrast, compound 13 showed the strongest binding affinity toward *S. aureus* DNA gyrase B (4URM) with a binding affinity of -7.94 kcal/mol, followed by compounds 14 (-7.83 kcal/mol), 5 (-7.82 kcal/mol), and 8 (-7.79 kcal/mol). These compounds exhibited more favorable binding affinities than chloramphenicol, suggesting stronger predicted interactions with the target proteins under the docking conditions employed.

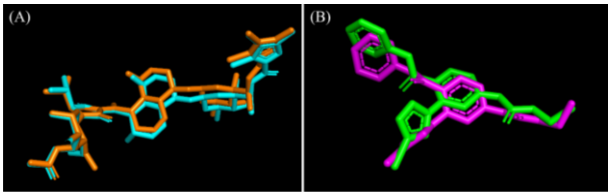

Figure 2. Superimposition of native and re-docked ligands: (A) *S. aureus* (4URM, RMSD = 0.710 Å) and (B) *E. coli* (6F86, RMSD = 1.566 Å)

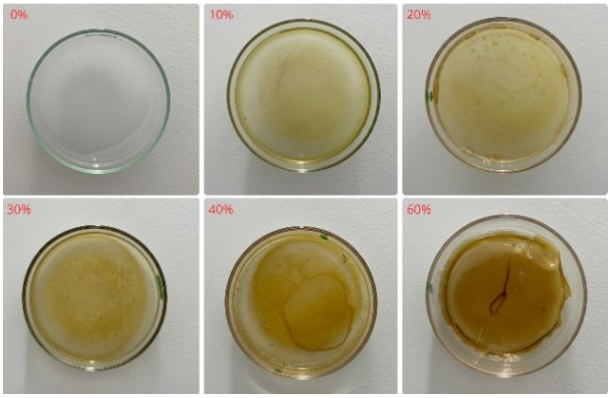

Figure 3. Formulation result

3.5. Edible Film Formulation

The increase in TOEE concentration in the film formulation (0–60%) led to noticeable changes in the visual characteristics of the edible films. The edible film without extract (0%) appeared clear, homogeneous, and transparent, reflecting the uniformly formed PVA-PEG polymer matrix without interference from other phases. This condition indicates good intermolecular compatibility and the absence of dispersed particles. At TOEE concentrations of 10–30%, the films began to exhibit a yellowish coloration while still remaining relatively homogeneous. At higher extract concentrations (40–60%), the edible films appeared darker and less homogeneous, with uneven surfaces and localized cracks, as shown in Figure 3.

3.6. Fourier Transform Infrared (FTIR) Characterization

FTIR analysis was employed to identify differences in functional groups and compound interactions in the edible films after incorporation of the extract. The FTIR spectra showed differences among TOEE, the polymer matrix without extract (EF0%), and the edible film containing 40% TOEE (EF40%). In EF40%, a peak appeared at 1078 cm^{-1}. (C-O alcohol/eter) [16], 3334 cm^{-1} (O–H) [16], and 1631 cm^{-1} (C=O) [17], as shown in Figure 4 and Table 6.

Table 5. Molecular docking scores of the identified compounds

Compound	6F86 (*E. coli*)	4URM (*S. aureus*)
	Binding affinity (kcal/mol)	
Chloramphenicol	-6.13	-6.89
Compound 1	-4.76	-4.60
Compound 2	-5.76	-5.66
Compound 3	-7.79	-7.29
Compound 4	-6.61	-6.60
Compound 5	-8.04	-7.82
Compound 6	-6.64	-7.40
Compound 7	-6.65	-7.42
Compound 8	-7.24	-7.79
Compound 9	-5.54	-5.52
Compound 10	-4.66	-5.09
Compound 11	-4.99	-5.33
Compound 12	-6.47	-6.98
Compound 13	-7.57	-7.94
Compound 14	-7.65	-7.83
Compound 15	-5.37	-5.12
Compound 16	-4.68	-5.59

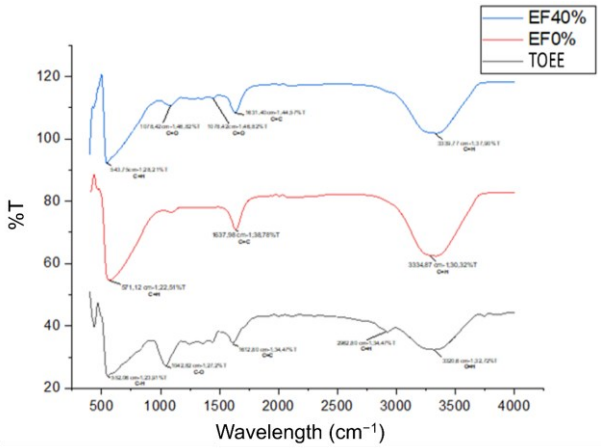

Figure 4. FTIR spectra

Table 6. FTIR spectral wavenumbers and corresponding functional groups

Wavenumber (cm^{-1})	Intensity (%T)	Functional group
3320.6	132.72	O-H
2962.0	134.47	C-H
1631.4	144.57	C=O
1012.8	134.47	C=C
1078.4	146.82	C-O
543.7	128.21	C-H
571.1	122.51	C-H
1837.9	138.78	C=O
3334.8	130.32	O-H
582.6	123.91	C-O

3.7. Scanning Electron Microscope (SEM) Characterization

SEM characterization showed that the TOEE-based edible films exhibited relatively smooth, homogeneous surfaces at low magnifications (500×–1000×), indicating a uniform distribution of film components. At higher magnifications (2000×–5000×), fine pores and small agglomerates were observed. These features may be associated with incomplete dispersion of the extract components. The formation of pores may also be attributed to solvent evaporation during drying. The film containing 40% TOEE exhibited a relatively homogeneous, porous surface, whereas increasing TOEE concentration tended to reduce surface smoothness.

As highlighted by the red circles in Figure 5, several agglomerated regions were observed on the edible film surface, indicating localized accumulation of TOEE within the PVA–PEG matrix. These agglomerates suggest that a small portion of the extract was not completely dispersed during film formation. The bright particles indicated by the red arrows in the 5000× micrograph represent extract-rich domains distributed around the agglomerated regions. These features indicate a heterogeneous distribution of TOEE at the microscopic level, contributing to increased surface roughness while maintaining the overall integrity of the film structure.

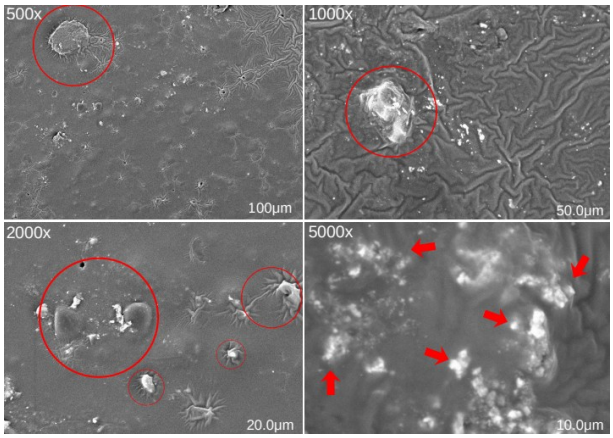

Figure 5. SEM images of the 40% TOEE-based edible film

3.8. Result of Thickness and Water Resistance

The results showed that the thickness of the edible films varied with increasing TOEE concentration. The film without extract (0% TOEE) had a thickness of 0.134 ± 0.015 mm, while the highest thickness was observed at 60% TOEE (0.250 ± 0.039 mm). However, the thickness did not increase consistently at intermediate concentrations; the lowest value was observed at 20% TOEE (0.112 ± 0.019 mm). The greater thickness at 60% TOEE may be related to the higher amount of extract incorporated into the film matrix, contributing additional solids to the film structure. Water absorption also varied among the TOEE concentrations, with the highest value at 10% TOEE (72.57%) and the lowest at 60% TOEE (27.11%). The values at 20%, 30%, and 40% TOEE were 57.27%, 65.34%, and 49.13%, respectively. The lower water absorption at higher TOEE concentrations may indicate changes in the interaction between the film matrix and water. The TOEE concentration influenced both the thickness and the water absorption of the edible films, as shown in Table 7.

Table 7. Thickness and water absorption of TOEE-based edible film

TOEE-based edible film concentration (%)	Thickness (mm) (Mean ± SD)	Water absorption (%)
0	0.134 ± 0.015	37.03
10	0.144 ± 0.015	72.57
20	0.112 ± 0.019	57.27
30	0.126 ± 0.015	65.34
40	0.188 ± 0.040	49.13
60	0.250 ± 0.039	27.11

Table 8. Tensile strength result

Sample (%)	MPa
0	28.46
40	30.70

Table 9. Comparison of the antibacterial activities of TOEE and TOEE-based edible films against *S. aureus* and *E. coli*

Concentration (%)	Inhibition zone (mm)			
	S. aureus		*E. coli*	
	TOEE	TOEE-based edible film	TOEE	TOEE-based edible film
10	10.00 ± 0.58	9.30 ± 0.58	8.67 ± 1.15	9.70 ± 0.58
20	12.33 ± 0.58	10.70 ± 0.58	11.00 ± 1.00	10.00 ± 0.58
30	12.33 ± 0.58	11.30 ± 0.58	11.67 ± 1.15	10.70 ± 0.00
40	13.67 ± 0.58	11.30 ± 1.15	13.33 ± 0.58	12.70 ± 0.58
60	13.33 ± 0.58	13.00 ± 0.00	13.00 ± 0.00	13.30 ± 0.58
Control (+)	25.33 ± 0.58	25.30 ± 0.58	23.67 ± 0.58	23.70 ± 0.58
Control (-)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data are presented as mean ± standard deviation (n = 3). Control (+): chloramphenicol; Control (-): DMSO.

3.9. Tensile Strength Result

Tensile strength testing was performed on the control edible film (0% extract) and the edible film containing 40% extract to evaluate the effect of TOEE incorporation on the mechanical properties of the film. The results showed that the tensile strength of the edible films increased after incorporating TOEE. The film without TOEE (0%) exhibited a tensile strength of 28.46 MPa, whereas the addition of 40% extract increased it to 30.7 MPa. This increase indicates that incorporating the TOEE influenced the film's mechanical properties, particularly its ability to withstand tensile forces. The results are presented in Table 8.

3.10. Antibacterial Activity Results

The antibacterial activities of TOEE and TOEE-based edible films against *S. aureus* and *E. coli* are presented in Table 9. Both TOEE and TOEE-based edible films exhibited antibacterial activity against both *S. aureus* and *E. coli* at all tested concentrations. In general, the inhibition zones increased with increasing extract concentration up to 40%, followed by a slight decrease or no substantial increase at 60%. This plateau may be attributed to reduced diffusion of the concentrated extract through the agar medium and a saturation effect, whereby further increases in extract concentration do not proportionally increase the inhibition zone. Compared with the pure extract, the edible films generally exhibited slightly smaller inhibition zones, possibly because incorporation of TOEE into the PVA–PEG matrix reduced the diffusion of bioactive compounds and promoted their gradual release while maintaining antibacterial activity.

3.11. Toxicity Screening Result

The toxicity test of the TOEE was conducted using *Artemia salina* larvae at concentrations ranging from 0 to 1000 ppm, with three replicates. The percentage of larval mortality was used to determine the LC_{50} value, with extracts having an LC_{50} below 1000 ppm categorized as toxic according to the BSLT criteria [18]. The test results showed an increase in larval mortality with increasing extract concentration, as shown in Figure 6; however, the obtained LC_{50} value of 1976 ppm indicated that the TOEE was classified as non-toxic and demonstrated preliminary safety potential for further studies.

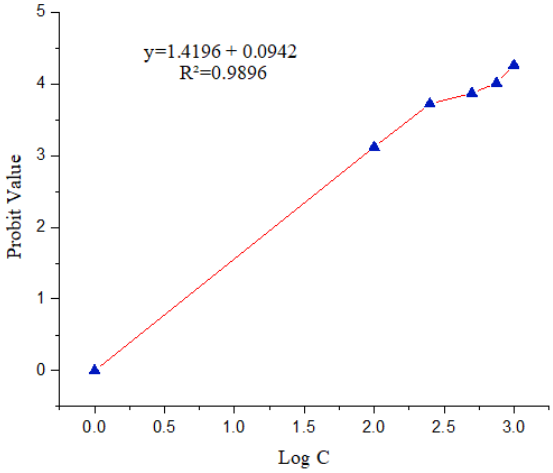

Figure 6. Toxicity screening curve of the TOEE

4. Conclusion

The results of this study demonstrated that TOEE contains various bioactive compounds with potential antibacterial activity, as confirmed by phytochemical screening and LC–MS characterization. The incorporation of TOEE into PVA–PEG-based edible films enhanced the functional properties of the films, including thickness, tensile strength, and antibacterial activity. Both the extract and TOEE-based edible films exhibited antibacterial activity against *E. coli* and *S. aureus*, while molecular docking suggested that several identified compounds possessed favorable binding affinities toward bacterial DNA gyrase B, supporting the observed antibacterial potential. Furthermore, TOEE exhibited low toxicity in the BSLT, with an LC_{50} value of 1976 ppm. Overall, TOEE-based edible films show promising potential as natural antibacterial active packaging materials for future food-packaging applications.

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