Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 175-186.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

IN VITRO ANTIMICROBIAL PHOTODYNAMIC ACTIVITY OF TECOMA STANS YELLOW FLOWERS EXTRACT LOADED IN NANO-EMULSION

Asmaa Y Khattab[1,2], Nagwa E Awad[3], Doaa Abdel Fadeel [*1], Maha Fadel[1]

[1]*Pharmaceutical Nano-Technology Unit, Department of Medical Applications of Laser, National Institute of Laser Enhanced Sciences (NILES), Cairo University, P.O. 12613, Giza, Egypt*
[2]*Mansheyet ElBakry Hospital, Ministry of Health and Population, Egypt*
[3]*Pharmacognosy Dept., National Research Centre, Dokki, Giza, Egypt*

Antimicrobial photodynamic therapy (aPDT) is an approach that was recently used to combat the resistance developed by pathogens toward antimicrobial drugs. Tecoma stans (L.) Jus., a prevalent tree in Egypt, was reported to be a promising photosensitizer in PDT. This study investigates the antimicrobial photodynamic activity of the methanolic crude extract of Tecoma stans (L.) Juss. ex Kunth loaded in a nano-emulsion as a natural photosensitizer. Molecular docking and molecular dynamics studies were conducted to investigate the binding between delphinidin as a promising lead and DNA's active site and to assess its stability through simulation The nano-emulsion formulation (TSFE-NE) was prepared, characterized, and evaluated for its antimicrobial photodynamic activities against three types of microorganisms: Gram-negative bacteria (Escherichia coli), Gram-positive bacteria (Staphylococcus aureus), and the pathogenic yeast Candida albicans. The percentage of microbial eradication was calculated after irradiation at 90 Mw/cm^2. The microbial eradication of TSFE-NE was enhanced after light exposure. Eradication percentages of 97.43%, 90.56%, and 87.77% were obtained for Escherichia coli, Staphylococcus aureus, and Candida albicans, respectively. These results confirmed the efficacy of natural photosensitizer and nanotechnology combination for improving antimicrobial photodynamic activities.

Keywords: *Tecoma stans flower extract; nano-emulsion; antimicrobial photodynamic therapy; natural photosensitizers; molecular dynamics*

INTRODUCTION

The main challenge in treating infectious diseases is the prevalence of resistance that microorganisms can rapidly develop against traditional antimicrobial therapy. Therefore, finding novel approaches to combat multidrug-resistant bacteria and fungi is pivotal [1]. Among these approaches is photodynamic therapy (PDT), a two-step approach depending on the excitation of a photosensitizer (PS) with light of a specific wavelength in the presence of tissue oxygen. By supplying oxygen and projecting the light onto the targeted lesion, the PS generates reactive oxygen species (ROS), ultimately destroying cells [2–5]. PDT is primarily utilized to treat localized malignant and non-malignant tumors. However, it has been reported that PDT holds significant promise in killing multidrug-resistant pathogenic microbes [2]. The ROS generated during PDT irreversibly destroys the microorganisms' DNA, cell wall, and biofilm[6, 7].

Antimicrobial photodynamic therapy (aPDT) effectiveness relies on the PS's characteristics. The good PS should have a suitable oxygen quantum yield, be hydrophilic, and be non-toxic without light. Several research groups have tested many plant extracts as a natural, affordable, and non-toxic PS source [8].

Received : 3/3/2025 & Accepted : 23/4/2025

*Corresponding author: Doaa Abdel Fadeel, E-mail: d_fadeel@cu.edu.eg or doaa.fadeel@niles.edu.eg

Tecoma stans (L.) Juss. is a prevalent tree in Egyptian gardens, adorned with countless blooms during the flowering season. The availability of these trees and their tremendous bloom output prompted the notion of finding a suitable biological activity. *Tecoma stans* has been used therapeutically as an antioxidant, antiproliferative, anti-diabetic, and antimicrobial agent [9–11].

Our previous study reported the active phytoconstituents of *Tecoma stans* crude flower extract (TSFE) and their photodynamic activity on cancer cell lines[10]. The results revealed the presence of anthocyanins (delphinidin and cyanidin) and many constituents that exerted antimicrobial activities in *Tecoma stans* crude flower extract. Those anthocyanins can be excited by light in the wavelength range of (400-450) and thus can be promising photosensitizers in photodynamic therapy[10]. In addition, delphinidin was reported to exert various activities as an anti-microbial, antioxidant, anti-inflammatory, anticancer, and neuroprotective agent[12]. Consequently, in the current work, molecular dynamics was employed to study the binding of delphinidin to the DNA complex system of the microorganisms. This gives us an idea about the efficacy of our crude extract phytoconstituents to be used as antimicrobial agents and know its eradication mechanism. Molecular dynamics is a computational method employed to model intricate systems at the atomic scale. Computer experiments enable us to obtain important kinetic and thermodynamic properties. Molecular dynamics simulations are commonly utilized to investigate the behavior of macromolecules, including nucleic acids and proteins [13].

Most phytoconstituents in plant extracts exhibit hydrophobic properties and tend to aggregate in aqueous environments. This aggregation impedes the pharmacokinetics, bioavailability, and, consequently, therapeutic efficacy. An important strategy for addressing these issues with phytomolecules' bioavailability is using nanotechnology[14]. Conjugation and/or loading of PS with nanoparticles can improve PS solubility and bioavailability. Moreover, nanoparticles can be tailored to achieve targeting of the PS to the desired tissues. Collectively, the combination of PS and nanotechnology can enhance the efficacy of aPDT [15].

Nanoemulsions have significant potential as nanocarriers for various plant extracts. These systems consist of an aqueous phase, surfactants/co-surfactants, and an oil phase. They are characterized by being homogeneous, thermodynamically stable, and isotropic [16].

This work highlights the effectiveness of combining aPDT, using a natural photosensitizer (TSFE), and pharmaceutical nanotechnology in the form of nanoemulsion (NE). The photodynamic anti-microbial activity of TSFE-NE was investigated against Gram-negative bacteria like *Escherichia coli* and Gram-positive bacteria like *Staphylococcus aureus*. Furthermore, the photodynamic antifungal activity of TSFE-NE was evaluated against *Candida albicans*. The chosen bacterial and fungal strains cause significant disease burden and are highly resistant to antimicrobial treatments.

Reviewing the previous literature, some publications have reported the antimicrobial activity of *Tecoma stans* [9, 10]. However, none have investigated the impact of *Tecoma stans* encapsulated in a nano-emulsion and evaluated its photodynamic activities against multiple strains in a single study.

MATERIALS AND METHODS

Materials

Isopropyl myristate (IPM) was purchased from Merck Co. in Germany. Transcutol and Tween 80 were purchased from Sigma Aldrich. The first culture medium, Nutrient Broth Medium (g/L), included Yeast extract (2.0 g), Peptone (5.0 g), Meat extract (1.0 g), and NaCl (5.0 g) with final pH 7.2 ± 0.2, was purchased from Lab. M Company in England. The second medium used was Nutrient Agar Medium (g/L), which included Yeast extract (2.0 g), Peptone (5.0 g), Meat extract (1.0 g), NaCl (5.0 g), and agar (20.0 g) with final pH 7.4 ± 0.2, was purchased from Fluka company in Spain.

Experimental

Collection of plant material and plant extract preparation

Tecoma stans flowers were gathered in August 2019 from El Merry Land Garden (Heliopolis, Cairo, Egypt). They were then identified at the National Research Centre in Egypt. The flowers were selected to prevent any interferences from chlorophylls.

To prepare TSFE, the fresh flowers (1.5 kg) were collected, washed, and cut into small pieces, then macerated with 3 L of methanol in the dark and repeated several times until complete exhaustion. The methanol extracts were filtered, collected, concentrated, and evaporated under reduced pressure using a rotavapor apparatus (Heidolph, Germany), and then stored at -20 °C. The resulting dried extract weighed 138 gm (9.2% of the weight of the fresh flowers).

Molecular Docking and Molecular Dynamic Simulation

A reverse pharmacophore mapping server estimated the binding properties of the TSFE-DNA.

The protein data bank provided the DNA crystal structure (PDB code: 453D), solved at 1.80 Å resolution. UCSF Chimera was used to prepare the structure, and PROPKA was employed to adjust the pH to 7.5 [17]. Chambiras Ultra 12.1 was utilized to create the 2D structure, while Avogadro software was employed to optimize the 2D structure for energy minimization using the steepest descent method along with MMFF94 force field [18].

Docking calculations were conducted via AutoDock Vina [19]. Gasteiger partial charges were assigned to the docking process. The AutoDock atom types were defined by the AutoDock graphical user interface (provided by MGL tools). For the grid box calculations, the dimensions were set to x = 11.14, y = 14.87, and z = 17.61, with the center grid parameters at x = 18.10, y = 12.99, and z = 18.72, and an exhaustiveness parameter of 8 was employed. Docked conformations were generated according to their docking energy using the Lamarckian genetic method in descending order [20].

The molecular dynamic simulations of every system were conducted through the GPU version of the PMEMD engine included in the AMBER 18 package [21, 22].

Preparation of *Tecoma stans* flower extract nanoemulsion (TSFE-NE)

To study the solubility of TSFE, 250 mg of TSFE was dissolved in equal amounts of olive oil and isopropyl myristate oil (IPM), followed by shaking for two hours in a water bath at 37 °C. This preliminary solubility test revealed that TSFE was easily soluble in IPM. Consequently, IPM was used as the oil phase for the nanoemulsion preparation (TSFE-NE). Tween 80 and transcutol were employed in a 1:1 ratio as the surfactant and co-surfactant, respectively [23].

The preparation of TSFE-NE involved the High-energy method [16]. To briefly explain, a mixture of 1 ml TSFE in IPM oil (8 mg/ml), tween 80, and transcutol was stirred for 15 minutes at 310 rpm. The mixture was then homogenized using an Ultraturrax T25 laboratory emulsifier (Ika, Staufen, Germany) at 9500 rpm. At the same time, distilled water was added dropwise at a controlled addition rate of 3.5 ml/min to complete the volume to 10 ml. The prepared emulsion was stored at room temperature (25 ± 2 ˚C) and shielded from light for 24 hours to allow for the emulsion stabilization, then refrigerated at 4 ˚C until further characterization.

Characterization of TSFE-NE

To assess the particle size distribution, zeta potential, and polydispersity index (PI) of TSFE-NE, a dynamic light scattering particle size analyzer (Zetasizer ZS, Malvern, UK) was utilized. TSFE-NE was diluted (1:25) with deionized water and sonicated for 10 minutes before the analysis. The measurements were repeated 3 months post-preparation to assess the stability of the prepared emulsion [24].

To analyze the morphology and structure of TSFE-NE, a high-performance, high-contrast transmission electron microscope (JEOL 1230, USA) with an operating voltage range of 40-120kV was used. A drop of the sample was mixed with water and applied to a carbon-coated grid. Next, it was treated with a 2% phosphotungstic acid solution for 30 seconds. After drying, the coated grid was placed on a slide and observed under the microscope. storage at 4°C

Assessment of Invitro Antimicrobial Photodynamic Activity

Assessment of MIC of TSFE-NE

The Minimum Inhibitory Concentration value (MIC) of TSFE-NE was assessed by applying the microdilution broth method_in pathogenic strains including (i) Gram-negative bacteria *Escherichia coli* (ATCC 25922), (ii) Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), and (iii) *Candida albicans* (ATCC 10231) as a pathogenic yeast.

The micro dilution broth method, take place using micro titer eliza plate, In this

method each well of the Eliza plate was filled with 175.0 µL of nutrient broth medium, then by serial dilution technique, each sample was used separately in first well by using the starting volume 100.0 µL that contains 80 µ g (concentration 0.8 mg/mL) of the used sample as a starting concentration, and by transfer of 100.0 µL from the first well to second well then transfer 100.0 µL from the second to the third, and so on to reach the final well that has sample concentration 1.25 µg of the used sample, Then the wells were inoculated with the tested pathogenic strains separately by transfer 25.0 µL [0.5 McFarland standard (1.5×10^8 CFU/ mL)] of tested strains, finally each well contain final volume 200 µL, then the inoculate 96 well plate incubate at 37°C for 24 hours, after the incubation period, the MIC was measured by the reading the absorption of the inoculated pales using Eliza reader, where the mixture was shaken vigorously for 20 seconds and allowed to stand, the absorbance was then measured at 600.0 nm using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). The lowest well absorbance reading of the previous culture mixture, which is equal to the absorption reading of the blank sample (nutrient broth medium only without any additives), is referred to as MIC activity[25].

Assessment of in vitro antimicrobial photodynamic activity of TSFE and TSFE-NE

The antimicrobial activities of TSFE that dissolved in DMSO (100 mg/ml), TSFE-NE, and empty NE (containing no TSFE) were investigated on pathogenic strains including (i) Gram-negative bacteria *Escherichia coli* (ATCC 25922), (ii) Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), and (iii) *Candida albicans* (ATCC 10231) as a pathogenic yeast.

The pathogen suspensions were prepared in nutrient broth media sterilized by autoclaving for 15 minutes at 121°C.

The pathogens were adjusted to a 0.5 McFarland standard containing 1.5 x 10^8 CFU/mL. Next, 15µl of each microbial suspension was inoculated into a 25 ml sterile nutrient broth medium (NB). 100 µl of each tested sample was incubated for 24 hours with the pathogen suspensions in an incubator shaker at 37 ºC and 120 rpm in the dark. Afterward, 100 µl of each sample was transferred to 6-well plates and irradiated for 15 minutes by a white lamp (Photon Scientific, Egypt) fitted with an optical filter that emits blue light (420 nm). The lamp's power was adjusted to deliver 100 mW/cm^2 at the plate's surface using a powermeter (Gentec-solo PE, Canada).

To measure the antimicrobial activities of the samples, the reduction in the number of microorganisms was calculated by comparing the colony-forming unit (CFU) of the treated strains to those in the control flask. Measurements were taken after 24 hours of incubation at 37 ºC in both dark and light conditions. The results were expressed as follows [25, 26]

The eradication (%) = (A- B /A) × 100

Where: A and B are the number of microorganisms in the control and the tested flasks, respectively.

RESULTS AND DISCUSSION

Molecular docking and molecular dynamic
Molecular dynamic and system stability

In our previous study, UPLC/MS/MS was conducted and the phytoconstituents in TSFE were identified. Among these identified phytoconstituents is delphinidin, one of the anthocyanin derivatives [10]. The molecular dynamic simulation was run to forecast the binding behavior of delphinidin to the DNA's active site and to assess its stability through simulations[21, 22].

The stability of the systems was evaluated in this study using Root-Mean-Square Deviation (RMSD) during the 14 ns simulations. A stable protein backbone atoms RMSD versus time is an indication of the near-equilibrium system. The RMSD between two sets of atomic coordinates is calculated as:

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(r_i - r_i^{\mathrm{ref}})^2}$$

Where:

N is the number of atoms being compared, ri is the position vector of atom i in the simulated structure, and ri ref is the position vector of atom i in the reference structure.

For the apo-DNA and delphinidin-complex systems, the average recorded RMSD

values were 2.23 ± 0.42 Å and 2.08 ± 0.46 Å, respectively (As shown in **Fig. 1A**). The findings demonstrated that delphinidin when bound to the DNA complex system, exhibited a relatively more stable conformation compared to the other investigated systems.

ROG was used to evaluate the compactness and stability of the system upon ligand binding[13]. The mean Rg values obtained for the complex systems of apo-DNA and delphinidin were 13.42 ± 0.20 Å and 13.32 ± 0.23 Å, respectively (**Fig. 1B**). Based on the observed behavior, the delphinidin-bound complex exhibited a very rigid structure against the DNA binding site domain.

By measuring the DNA's solvent-accessible surface area (SASA), the compactness of the hydrophobic core of the DNA was investigated. This was accomplished by measuring the DNA's solvent-visible surface area, which is crucial for the stability of biomolecules. According to **Fig. 1C**, the average SASA values for the delphinidin-complex systems and apo-DNA were 4333.12 Å and 4399.40 Å, respectively. Together with the results from the RMSD and ROG calculations, the SASA finding demonstrated that the delphinidin complex system is still present inside the DNA association domain binding site.

Binding free energy calculation and binding interaction mechanism

The binding free energies were determined using AMBER18's MM-GBSA software. As demonstrated in **Table 1**, except ΔGsolv, all reported computed energy components yielded high negative values indicating beneficial interactions. The findings showed that the delphinidin-complex systems' binding affinity was -32.97 kcal/mol.

The results demonstrate that the larger positive electrostatic energy component drives the interactions between the ligand molecules and the DNA association domain protein receptor residues **Table 1**.

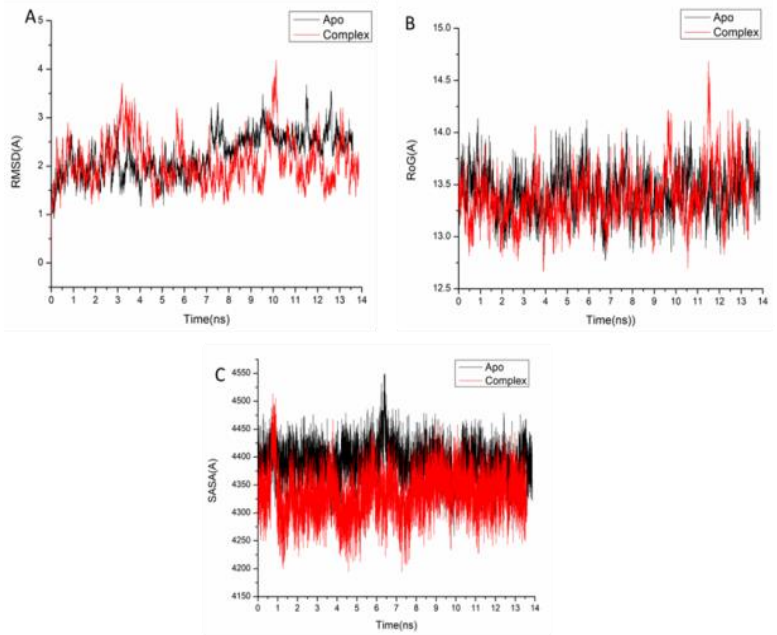

Fig. 1: [A] RMSD of the DNA backbone atoms. [(B) ROG of DNA residues; (C) solvent accessible surface area (SASA) of the backbone atoms relative (black) to the starting minimized over 14 ns for the Catalytic domain binding site of DNA with delphinidin complex system (red).

Table 1: The calculated energy binding for delphinidin against the DNA association domain binding site receptor.

Energy Components (kcal/mol)					
Complex	**ΔE_{vdW}**	**ΔE_{elec}**	**ΔG_{gas}**	**ΔG_{solv}**	**ΔG_{bind}**
delphinidin	-36.23± 0.22	-56.94± 0.12	-60.17 ± 0.14	72.20± 0.95	-32.97± 0.24

ΔEvdW: van der Waals energy; ΔEelec: electrostatic energy; ΔGsolv : solvation-free energy; ΔGbind : calculated total binding free energy.

Identification of the critical residues responsible for ligand binding

From **Fig. 2**, the significant positive impact of delphinidin on the DNA domain binding site receptor is primarily observed from specific residues DG2 (-0.86 kcal/mol), Gln 26(-1.44 kcal/mol), Ser 29 (-0.927 kcal/mol), Phe30 (-0.579 kcal/mol), DG4 (-0.307 kcal/mol), DA5 (-0.994 kcal/mol), DA6 (-2.683kcal/mol), DT7 (-1.607kcal/mol), DT8 (-0.303kcal/mol), DT19 (-0.382 kcal/mol), DT20 (-3.057kcal/mol), DC21 (-2.883 kcal/mol), DG22(-2.816 kcal/mol), and DC23 (-0.586 kcal/mol).

Preparation and characterization of TSFE-NE

TSFE-NE was successfully created using IPM as the oil phase and distilled water as the external water phase. Tween 80 was employed as a surfactant, while transcutol was added as a co-surfactant. IPM is known to have excellent penetration-enhancing properties [27]. Tween 80 is a non-ionic surfactant, commonly used in nanoemulsion formation It lowers the interfacial tension and causes oil droplet disruption reduction. However, Tween 80 alone cannot effectively reduce the surface tension, so it is usually mixed with a co-surfactant like transcutol [28]. Transcutol is a frequently used co-surfactant in nano-emulsion formulations due to its high miscibility in nonpolar solvents and ability to enhance cell membrane penetration [29]. Moreover, it aids the dispersion process by reducing the interfacial tension and induces a flexible film [30, 31].

The prepared nanoemulsion displayed a spherical shape with minimum aggregation, as revealed by the TEM image (**Fig. 3**), with a particle size of 124.5±32.83 nm (**Table 2**). Moreover, the prepared formula exhibited a zeta potential value of -26.2±8.18, as shown in **Table 2**. These values promote good repulsion between the nano-emulsion droplets, ultimately lowering aggregation and increasing the colloidal stability of the nano-emulsion [32]. After being stored for three months at 4 ˚C, the nano-emulsion phases showed no signs of separation upon visual inspection. However, there was a slight increase in particle size and a decrease in zeta potential (**Table 2**).

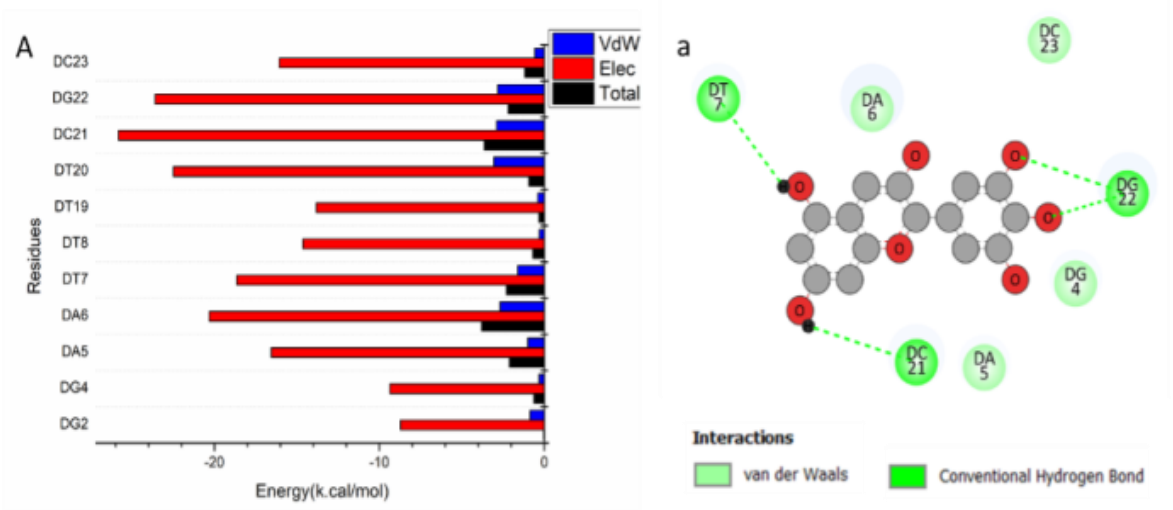

Fig. 2: Per-residue decomposition plots showing the energy contributions to the binding and stabilization of delphinidin into the DNA catalytic binding site.

Table 2: Composiion and characterization of the prepared TSFE-NE.

Formula	Particle Size (nm)	Particle size (nm) after 3 months	PI	PI after 3 months	Zeta potential (mV)	Zeta potential (mV) after 3 months
TSFE-NE	124.5±32.83	212±33.79	0.4	0.592	-26.2±8.18	-14.9±3.83

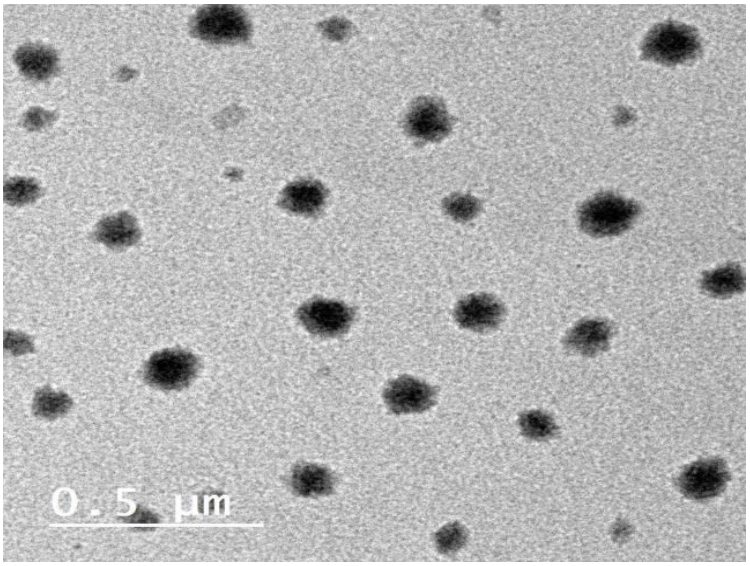

Fig. 3: TEM photo of the formulated TSFE-NE.

Assessment of in vitro antimicrobial photodynamic activity of TSFE and TSFE-NE

The MIC of TSFE-NE on *Escherichia coli, Staphylococcus aureus, and Candida albicans* was reported to be 200, 300, 250 µg/mL, respectively.

The antimicrobial photodynamic activities of TSFE, TSFE-NE, and empty nano-emulsion as control (C) were tested against various microorganisms before and after light exposure. The poor solubility of TSFE and low bioavailability were overwhelmed by the preparation of nano-emulsion as a nano-delivery system for TSFE. The first tested microorganism is the gram-negative bacteria *Escherichia coli.* Though commonly found in the gastrointestinal tract of animals and humans, certain strains of *Escherichia coli* are harmful and can cause severe gastrointestinal and urinary tract infections.

Multidrug resistance of *Escherichia coli* is becoming a growing concern in both human and veterinary medicine. In this study, using nanoemulsion combined with aPDT could potentiate the eradication of Gram-negative *Escherichia coli*. **Fig. 4** illustrates that the eradication of *Escherichia coli* treated by TSFE did not show a significant difference before and after irradiation. Treating the *Escherichia coli* by TSFE-NE resulted in a significant bacterial eradication compared to the crude extract. After irradiation, the bacterial eradication was 97.43%.

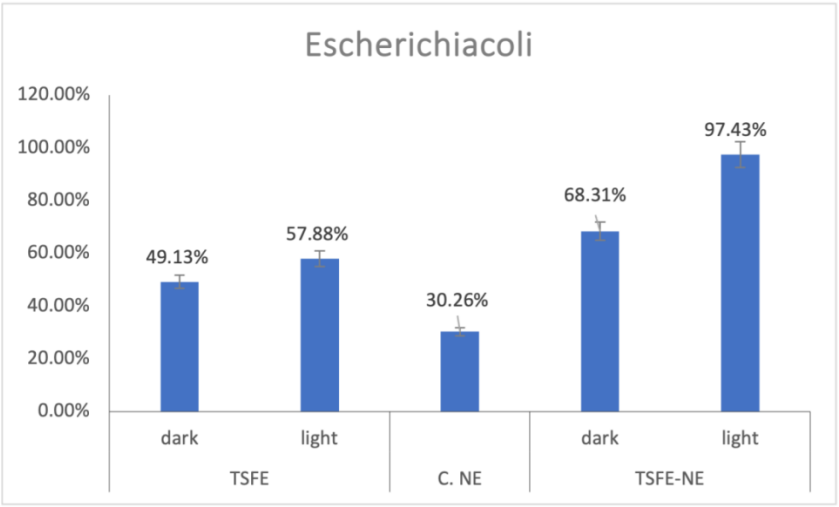

Fig. 4: The % of microbial eradication of *Escherichia coli* upon treatment with TSFE and TSFE-NE in the dark and after light exposure.

Another tested pathogen is the gram-positive bacteria *Staphylococcus aureus*. It is one of the major pathogens that causes a variety of serious infections in humans. It can develop resistance against antibiotics, potentially causing rapidly spreading infections. As illustrated in **Fig. 5**, TSFE-NE showed significant potentiation of *Staphylococcus aureus* eradication after light irradiation. Consequently, aPDT treatment using TSFE-NE could introduce a highly effective treatment that can combat bacterial resistance.

The antifungal aPDT activity of TSFE was also tested against *Candida albicans*. Candidiasis is an infection caused by many types of Candida species, with *Candida albicans* being the most common. The disease can affect the skin, mucous membranes, or internal organs. To treat these infections, different classes of antifungal drugs, such as azoles, polyenes, and echinocandins, have been used. However, the development of drug resistance can lead to therapeutic failure. **Fig. 6** showed that TSFE-NE significantly potentiated the antifungal activity of TSFE. There was a non-significant difference before and after light irradiation of TSFE-NE because of the high activities of the nanoforms that masked the photosensitizer action. Therefore, TSFE-NE is a good choice for *Candida albicans* eradication.

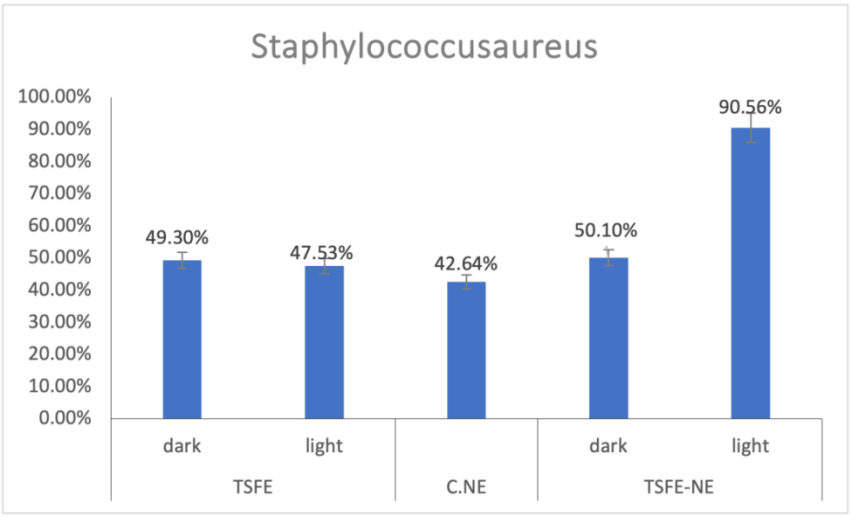

Fig. 5: The % of microbial eradication of *Staphylococcus* aureus upon treatment with TSFE and TSFE-NE in the dark and after light exposure.

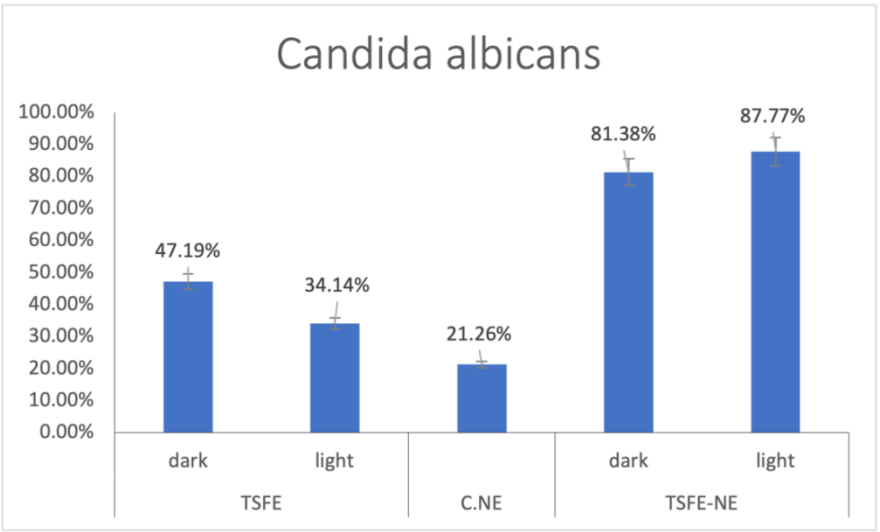

Fig. 6: The % of microbial eradication of *Candida albicans* upon treatment with TSFE and TSFE-NE in the dark and after light exposure.

The results revealed that TSFE and TSFE-NE were shown to decrease bacterial survival, indicating their activities against bacteria. The nanoemulsion was reported to improve the efficacy of the herbal bio-constituents by enhancing their solubility, bioavailability, and absorption while lowering the required doses and associated adverse effects[16]. Moreover, nanoemulsion may disrupt the cell membrane of the microbial cell and inhibit the budding process, which in turn is due to the destruction of the membrane integrity [33].

In addition, it was suggested that aPDT can induce micro-damages to the cell envelope, causing an increase in the cell membrane permeability. Photosensitization enhances the susceptibility of the bacterial cell to treatment, making it a promising alternative for combating resistant bacteria [34].

Previous studies confirmed that TSFE contains phytoconstituents with antibacterial and antifungal activities such as Isorhamnetin, kaempferol, Luteolin, Succinic acid, delphinidin, and Esculin[10, 35]. Moreover, Bakr *et al*., 2019 [36] reported that the *Tecoma stans* leaf methanolic extract contains phytoconstituents that exhibited antimicrobial activities against several bacterial and fungi strains. Tariq H. *et al* [11] conducted a study on the antibacterial potential of *Tecoma stans* extracts of leaves, branches, and silver nanoparticles (AgNPs). According to the results, AgNPs demonstrated more significant antibacterial activity than crude extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) and sensitive *Staphylococcus aureus* with different strains.

Regarding the photodynamic activity of *Tecoma stans*, Khattab *et al* [10] studied the photodynamic activities against cancerous cell lines. However, almost no studies have investigated the antimicrobial photodynamic activities of TSFE and TSFE-NE in one such study.

The mechanism of PDT is oxygen-dependent. A photochemical reaction occurs when a photosensitizing compound is activated by light, resulting in the generation of cytotoxic reactive oxygen species, primarily singlet oxygen. These species can damage cell membranes and cause irreversible harm to biological systems [37].

Our findings indicate that using natural phytochemical components, nanotechnology, and photodynamic therapy together can improve the killing of microbes, which is a significant step forward considering the global issue of multidrug-resistant infectious agents. Hence, a crucial medical breakthrough is discovering a natural, new, potent, and relatively non-toxic antimicrobial agent. To enhance the effectiveness and safety of drugs, new techniques, and pharmaceutical forms can be developed to improve drug delivery to target sites.

Conclusion

Microbial resistance to antibiotics has emerged as a significant problem in medicine in recent years. Combining photodynamic therapy with nanotechnology can be a promising treatment modality for resistant bacterial and fungal infections. This is because nanotechnology and photodynamic therapy have proven to be highly effective in exploiting the many biological activities of phytochemical components. The results of this study are promising and should be extended to investigate other bacterial models. Also, it highlighted the importance of natural products as a source of biologically active compounds. Their activities can be proven theoretically and practically via molecular dynamics and microbiological analysis.

REFERENCES

1. L. Serwecińska, "Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health", ***Water (Basel)***, 12(12), 3313 (2020).
2. M. Awad, N. Thomas, T. J. Barnes and C. A. Prestidge, "Nanomaterials enabling clinical translation of antimicrobial photodynamic therapy", ***J Control Release***, 346, 300–316 (2022).
3. S. Dingiswayo, K. Burgess, B. Babu, J. Mack and T. Nyokong, "Photodynamic Antitumor and Antimicrobial Activities of Free-Base Tetra (4-methylthiolphenyl) chlorin and Its Tin (IV) Complex", ***Chempluschem***, 87(5), e202200115 (2022).
4. E. Dube, D. O. Oluwole, N. Nwaji and T. Nyokong, "Glycosylated zinc phthalocyanine-gold nanoparticle conjugates for photodynamic therapy: Effect of nanoparticle shape", ***Spectrochim Acta A Mol Biomol Spectrosc***, 203, 85–95 (2018).

5. L. Ulfo, P. E. Costantini, M. Di Giosia, A. Danielli and M. Calvaresi, "EGFR-Targeted Photodynamic Ther-apy", ***Pharmaceutics***, 14(2), 241 (2022).
6. E. M. Abdullah, A. Tawfik, M. Fadel, J. Alsharnoubi, D. A. A. Fadeel and N. Abdallah, "Photodynamic therapy of tinea capitis in children using curcumin loaded in nanospanlastics: A randomized controlled comparative clinical study", ***J Drug Deliv Sci Technol***, 103496 (2022).
7. J. M. de Almeida, H. R. Matheus, B. E. Sendão Alves, D. J. Rodrigues Gusman, M. J. H. Nagata, E. M. de Abreu Furquim and E. Ervolino, "Evaluation of antimicrobial photodynamic therapy with acidic methylene blue for the treatment of experimental periodontitis***", PLoS One***, 17(2), e0263103 (2022).
8. R. B. Villacorta, K. F. J. Roque, G. A. Tapang and S. D. Jacinto, "Plant extracts as natural photosensitizers in photodynamic therapy: in vitro activity against human mammary adenocarcinoma MCF-7 cells", ***Asian Pac J Trop Biomed***, 7(4), 358–366 (2017).
9. M. Anand and R. Basavaraju, "A review on phytochemistry and pharmacological uses of *Tecoma stans* (L.) Juss. ex Kunth", ***J Ethnopharmacol,*** 265, 113270 (2021).
10. A. Y. Khattab, N. E. Awad, D. A. Fadeel and M. Fadel, "Dual antineoplastic and photodynamic effects of methanolic extract of *Tecoma stans* yellow flowers for cancer treatment", ***J Herbmed Pharmacol***, 12(3), 432–441 (2023).
11. H. Tariq, M. Rafi, M. I. Amirzada, S. A. Muhammad, M. A. Yameen, A. Mannan, T. Ismail, I. Shahzadi, G. Murtaza and N. Fatima, "Photodynamic cytotoxic and antibacterial evaluation of *Tecoma stans* and Narcissus tazetta mediated silver nanoparticles", ***Arab J Chem***, 15(3), 103652 (2022).
12. M. Yuan, Q. He, W. Xiang, Y. Deng, S. Lin and R. Zhang, "Natural compounds efficacy in Ophthalmic Diseases: A new twist impacting ferroptosis", ***Biomed Pharmacother***, 172, 116230 (2024).
13. D. A. Fadeel, M. Fadel, A. I. El-Kholy, A. A. El-Rashedy, E. Mohsen, M. I. Ezzat and M. Y. Issa, "Nano-Liposomal Beetroot Phyto-Pigment in Photodynamic Therapy as a Prospective Green Approach for Cancer Management: In Vitro Evaluation and Molecular Dynamic Simulation", ***Pharmaceutics***, 16(8),1038 (2024).
14. P. K. Mukherjee, R. K. Harwansh and S. Bhattacharyya, "Bioavailability of herbal products: approach toward improved pharmacokinetics", ***in Evidence-Based Validation of Herbal Medicine***, 217–245 (2015).
15. S. Kwiatkowski, B. Knap, D. Przystupski, J. Saczko, E. Kędzierska, K. Knap-Czop, J. Kotlińska, O. Michel, K. Kotowski and J. Kulbacka, "Photodynamic therapy–mechanisms, photosensitizers and combinations", ***Biomed Pharmacother***, 106, 1098–1107 (2018).
16. R. K. Harwansh, R. Deshmukh and M. A. Rahman, "Nanoemulsion: Promising nanocarrier system for delivery of herbal bioactives", ***J Drug Deliv Sci Technol***, 51, 224–233 (2019).
17. H. Li, A. D. Robertson and J. H. Jensen, "Very fast empirical prediction and rationalization of protein pKa values", ***Proteins***, 61(4), 704–721 (2005).
18. S. S. Ragab, N. E. Ibrahim, M. S. Abdel-Aziz, A. A. Elrashedy and A. K. Allayeh, "Synthesis, biological activity, and molecular dynamic studies of new triazolopyrimidine derivatives", ***Results Chem***, 6, 101163 (2023).
19. O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading***", J Comput Chem***, 31(2), 455–461 (2010).
20. F. Stanzione, I. Giangreco and J. C. Cole, "Use of molecular docking computational tools in drug discovery", ***Prog Med Chem***, 60, 273–343 (2021).
21. M. Hasanin, A. H. Hashem, A. A. El-Rashedy and S. Kamel, "Synthesis of novel heterocyclic compounds based on dialdehyde cellulose: characterization, antimicrobial, antitumor activity, molecular dynamics simulation and target identification", ***Cellulose***, 28(13), 8355–8374 (2021).
22. S. Mirzaei, F. Eisvand, F. Hadizadeh, F. Mosaffa, A. Ghasemi, R. Ghodsi, "Design, synthesis and biological evaluation of novel 5, 6, 7-trimethoxy-N-aryl-2-styrylquinolin-4-amines as potential anticancer agents and tubulin

polymerization inhibitors", ***Bioorg Chem***, 98, 103711 (2020).
23. N. Aly, A. Husseein, H. Emam and G. Rashed, "Curcuma longa extract nanoemulsion preparation and demonstration of its in vitro activity against Schistosoma mansoni cercariae, schistosomules and adult stages", ***J Parasitol***, 10(1-2), 44–51 (2017).
24. L. F. Dalmolin and R. F. V Lopez, "Nanoemulsion as a platform for iontophoretic delivery of lipophilic drugs in skin tumors", ***Pharmaceutics***, 10(4), 214 (2018).
25. A. A. El-Anssary, G. F. A. Raoof, D. O. Saleh and H. M. El-Masry, "Bioactivities, physicochemical parameters and GC/MS profiling of the fixed oil of Cucumis melo L seeds: A focus on anti-inflammatory, immunomodulatory, and antimicrobial activities", ***J Herbmed Pharmacol***, 10(4), 476–485 (2021).
26. F. A. Mostafa, A. A. Abd El Aty, E. R. Hamed, B. M. Eid and N. A. Ibrahim, "Enzymatic, kinetic and anti-microbial studies on Aspergillus terreus culture filtrate and Allium cepa seeds extract and their potent applications", ***Biocatal Agric Biotechnol***, 5, 116–122 (2016).
27. A. Eichner, S. Stahlberg, S. Sonnenberger, S. Lange, B. Dobner, A. Ostermann, T. E. Schrader, T. Hauß, A. Schroeter and D. Huster, "Influence of the penetration enhancer isopropyl myristate on stratum corneum lipid model membranes revealed by neutron diffraction and 2H NMR experiments", ***Biochim Biophys Acta, Biomembr***, 1859(5), 745–755 (2017).
28. A. Tarik Alhamdany, A. M. H. Saeed and M. Alaayedi, "Nanoemulsion and Solid Nanoemulsion for Improving Oral Delivery of a Breast Cancer Drug: Formulation, Evaluation, and a Comparison Study", ***SPJ***, 29(11), 1278–1288 (2021).
29. K. M. Hosny, N. A. Alhakamy and K. S. Al Nahyah, "The relevance of nanotechnology, hepato-protective agents in reducing the toxicity and augmenting the bioavailability of isotretinoin", ***Drug Deliv,*** 28(1), 123–133 (2021).
30. J. Akhtar, H. Hussain Siddiqui, S. Fareed and M. Aqil, "Nanomulsion as a carrier for efficient delivery of metformin", ***Curr Drug Deliv***, 11, 243–252 (2014).
31. P. S. Prasad, S. S. Imam, M. Aqil, Y. Sultana and A. Ali, "QbD-based carbopol transgel formulation: characterization, pharmacokinetic assessment and therapeutic efficacy in diabetes", ***Drug Deliv,*** 23(3), 1047–1056 (2016).
32. G. Kumar, T. Virmani, K. Pathak, O. Al Kamaly and A. Saleh, "Central Composite Design Implemented Azilsartan Medoxomil Loaded Nanoemulsion to Improve Its Aqueous Solubility and Intestinal Permeability: In Vitro and Ex Vivo Evaluation", ***Pharmaceuticals***, 15(11), 1343 (2022).
33. M. Fadel, M. Nasr, R. M. Hassan and S. S. Thabet, "Cationic zinc (II) phthalocyanine nanoemulsions for photodynamic inactivation of resistant bacterial strains", ***Photodiagnosis Photodyn Ther***, 34, 102301 (2021).
34. A. S. Garcez, M. Kaplan, G. J. Jensen, F. R. Scheidt, E. M. Oliveira and S. S. Suzuki, "Effects of antimicrobial photodynamic therapy on antibiotic-resistant Escherichia coli", ***Photodiagnosis Photodyn Ther***, 32, 102029 (2020).
35. A. Husain, H. Chanana, S. A. Khan, U. M. Dhanalekshmi, M. Ali, A. A. Alghamdi and A. Ahmad, "Chemistry and pharmacological actions of delphinidin, a dietary purple pigment in anthocyanidin and anthocyanin forms", ***Front Nutr***, 9, 746881 (2022).
36. R. O. Bakr, M. A. A. Fayed, M. A. Salem and A. S. Hussein, "*Tecoma stans*: alkaloid profile and antimicrobial activity", ***J Pharm Bioallied Sci,*** 11(4), 341–347 (2019).
37. E. Polat and K. Kang, "Natural photosensitizers in antimicrobial photodynamic therapy", ***Biomedicines***, 9(6), 584 (2021).

Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 1, 2025, pp. 175-186.

نشرة العلوم الصيدليـــة
جامعة أسيوط

BULL. PHARM. SCI. Assiut Univ.

النشاط الضوئي الديناميكي المضاد للميكروبات لمستخلص أزهار تيكوما ستانس الصفراء المحمل في مستحلب نانوي (دراسة مختبرية)

أسماء خطاب[1،2] – نجوي عوض[3]– دعاء أحمد عبد الفضيل*[1] – مها فاضل[1]

[1]وحدة التكنولوجيا الصيدلية، قسم تطبيقات الليزر الطبية، المعهد القومي لعلوم الليزر، جامعة القاهرة، جمهورية مصر العربية

[2]مستشفى منشية البكري، وزارة الصحة والسكان، جمهورية مصر العربية

[3] قسم العقاقير، المركز القومي للبحوث، جمهورية مصر العربية

يُعد العلاج الضوئي الديناميكي المضاد للميكروبات (aPDT) أحد الأساليب الحديثة المستخدمة لمواجهة مقاومة الميكروبات للعلاجات التقليدية. وتُشير الدراسات إلى أن.Tecoma stans (L.) Jus ، وهي شجرة شائعة في مصر، تُعد من المحسسات الضوئية الواعدة في هذا المجال. تهدف هذه الدراسة إلى تقييم الفعالية الضوئية الديناميكية المضادة للميكروبات لمستخلص Tecoma stans (L.) Juss. Kunth ex الخام الميثانولي، بعد تحميله في مستحلب نانوي، ليعمل كمحسس ضوئي طبيعي.

ولفهم آلية التأثير، تم إجراء دراسات الالتحام الجزيئي والديناميكيات الجزيئية لاستكشاف مدى ارتباط مركب الدلفينيدين الذي يُعدّ مرشحًا واعدا بالموقع النشط في الحمض النووي(DNA) ، بالإضافة إلى تقييم استقراره من خلال المحاكاة الحاسوبية.

كما تم تحضير المستحلب النانونية المحمل بالمستخلص النباتي (TSFE-NE) وتوصيفه ثم اختبار فعاليته الضوئية الديناميكية المضادة للميكروبات ضد ثلاثة أنواع من المكروبات:

- البكتيريا سالبة الجرام: Escherichia coli
- البكتيريا موجبة الجرام:Staphylococcus aureus
- فطر الخميرة الممرض:Candida albicans

بعد تعريض المستخلص للإشعاع الضوئي بقدرة ٩٠ ميللي وات/سم^2، لوحظ تحسّن ملحوظ في فعاليته المضادة للميكروبات. حيث بلغت نسب الإبادة:

- ٩٧,٤٣ % للبكتيريا.Escherichia coli
- ٩٠,٥٦ % للبكتيريا.Staphylococcus aureus
- ٨٧,٧٧ % لفطر.Candida albicans

تؤكد هذه النتائج أن الجمع بين المحسسات الضوئية الطبيعية وتقنيات النانو يُمكن أن يُعزز بشكل كبير من الفعالية الضوئية الديناميكية المضادة للميكروبات، مما يفتح آفاقًا جديدة لتطوير علاجات مبتكرة وفعالة ضد الممرضات المقاومة للأدوية التقليدية.