

## Preparation and Characterization of Antimicrobial Vanillin-Modified Polyurethane Composite

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### Abstract

Modification of polyurethane foams composite by vanillin and thiourea is a search for a new antimicrobial active composite. TPU/Vanillin, a polyurethane foam composite loaded with vanillin and thiourea, was produced and tested for antimicrobial activity. TPU/Vanillin composite was synthesized by combining the surface qualities of polyurethane foams with the biological functions of thiourea and vanillin. TPU/Vanillin composite was identified by IR, UV-Vis, bandgap energy, magnetic susceptibility, chemical stability and pH<sub>PZC</sub>. The antibacterial and antifungal properties of TPU/Vanillin composite were investigated. TPU/Vanillin composite was tested *in vitro* against Gram-negative bacteria, *Escherichia coli*, Gram-positive bacteria, *Bacillus cereus*, and fungus, *Aspergillus niger*. Agar well diffusion, minimum inhibition concentration (MIC) and minimum microbicidal concentration (MMC) tested were used to determine the sensitivity of the tested microbes to the prepared composite. Zones of inhibition of TPU/Vanillin were  $16 \pm 0.12$ ,  $15 \pm 0.19$  and  $25 \pm 0.20$  mm against *E. coli*, *B. cereus*, and *A. niger*, respectively. Successfully inhibiting the growth of microbial strains with MIC values of 130, 140 and 40  $\mu\text{g/ml}$  against *E. coli*, *B. cereus*, and *A. niger*, respectively, proved that TPU/Vanillin composite could be further improved for potential applications such as food packaging and coating.

**Keywords:** Vanillin, polyurethane foam, thiourea, characterization, MIC, antimicrobial.

### Introduction

Foodborne contaminations have been identified as the leading cause of illnesses and even deaths caused by bacteria or fungi entering the body through contaminated food (Espitia et al., 2016). Food contamination can occur because

of food exposure to a contaminated environment during processing or packaging. Food spoilage because of pathogen contamination is not only harmful to consumers but also causes significant economic losses to manufacturers. Antibiotic resistance is now the most important worldwide challenge to successful bacterial and fungal infection therapy (Nwobodo et al., 2022). Bacterial drug

resistance might be produced by permeability changes in the cell membrane, as well as specific enzymes that can block the efficacy of antimicrobial drugs (Wang et al., 2017). Biocompatible, non-toxic, readily soluble, efficient, and hypoallergenic antifungal drugs should be employed in medicinal applications (Ribeiro et al., 2021). In hospitals, patients are more vulnerable to fungal and bacterial diseases. The most prevalent fungal infections are aspergillosis, invasive candidiasis, and mucormycosis (Gurunathan et al., 2022). Some of the most difficult difficulties in modern medicine are the growing resistance of fungal diseases to present medications and the absence of alternative and effective treatment alternatives. The most critical variables for effective antifungal medication are early and correct diagnosis, followed by treatment before resistance issues develop in, limiting and complicating treatment alternatives. Furthermore, surgical excision of all diseased tissues, as well as adjuvant therapy, are the most efficient methods of eradicating mucormycosis (Fang et al., 2023; Spellberg et al., 2005). As a result, research into novel safe and effective antimicrobial drugs that might be used in a variety of relevant disciplines continues (Nayaka et al., 2021).

The mechanical resistance supplied by the cell wall and membrane keeps microbial cells in form. The cell wall is made up of three primary macromolecules (mannoproteins, -glucan, and chitin) that are required for cell shape sustainability and mechanical damage prevention (Zhao et al., 2022). The integrity of fungal cell membranes, which are made up of proteins, phospholipids, and sugars, is critical for fungus survival (Zhao et al., 2022). The antibacterial and antifungal mechanisms of an antimicrobial agents are dependent on the particular interaction with the surface and metabolism of agents into the microbe (Ansari et al., 2013). Many antimicrobial medications can also impair fungi's cellular antioxidation mechanism, making them oxidative stress agents (Kim et al., 2011). Destructive effects of inhibitory drugs against certain bacteria manifest as cell wall rupture, resulting in release of cytoplasmic contents and shrinkage of cytoplasmic size. Deeper impacts may include the coagulation of cell proteins, the formation of mineral crystals owing to cytoplasm dehydration, and the full discharge of cytoplasmic contents (Alamri et al., 2012).

*Bacillus cereus*, *Escherichia coli* and *Aspergillus niger* are examples of the most common resistant microbial strains. *B. cereus* is a food pathogen that causes epidemics of food poisoning with vomiting. Because *B. cereus* is difficult to eliminate using traditional procedures and techniques, it poses a significant hazard to food safety and human health (Krzepiko et al., 2023). *E. coli* is a food pathogenic and toxic bacterium that causes food deterioration and, when consumed, various health-threatening consequences. It can cause nausea, vomiting, stomach cramps, diarrhea, tiredness, and fever (Ma et al., 2023). *A. niger* is a fungus that can cause deadly diseases known as aspergillosis. *A. niger* is a pathogen fungus that mostly infects immunocompromised people (Jahani & Aminifard, 2020; Latgé, 1999). Mycotoxins and aflatoxins are produced by *A. niger*, a widespread food contamination (Navale et al., 2021; Sheikh-Ali et al., 2014).

Polyurethane foams (PUFs) are primarily employed in a variety of industrial and medical applications. Because of their chemical composition and comfort properties, PUFs are replacing older polymers (Yang et al., 2019). Functional PUFs may benefit from both functional groups and the polymeric structure of PUFs (Alamri et al., 2012).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a phenolic aldehyde that is the biologically active element in vanilla and possesses antibacterial properties. It is also utilized as a flavoring additive in food, drinks, and fragrances (Maisch et al., 2022). Vanillin is abundant in vanilla beans and *Vanilla planifolia*, which have long been used in traditional medicine for its medicinal powers (Ribeiro et al., 2021). Vanillin is widely regarded as a risk-free reagent having antibacterial, antioxidant, and anti-carcinogenic properties. Furthermore, its principal components are employed as food preservatives (Maisch et al., 2022). Also, thiourea derivatives have been demonstrated to exhibit bactericidal and fungicidal properties due to the presence of nitrogen and sulfur (El-Nour et al., 2023). Thiourea derivatives shown increased antibacterial activity as well as antifungal activity (Limban et al., 2018).

Thus, the goal of this research is to synthesize, characterize, and analyze TPU/Vanillin's antimicrobial action. TPU/Vanillin composite was tested for antibacterial effectiveness

against *E. coli* and *B. cereus* bacteria. TPU/Vanillin composite was also evaluated for antifungal effectiveness against the *A. niger* fungus. TPU/Vanillin's antibacterial activity was assessed using the agar well diffusion, minimum inhibition concentration and minimum microbicidal concentration techniques.

## Materials and Methods

### Materials and reagents

**TPU/Vanillin:** Commercial PUF sheets ( $d = 12 \text{ kg/m}^3$ ) were supplied by the Egyptian company for foam production (Foamex) in New Damietta. Vanillin ( $\text{C}_8\text{H}_8\text{O}_3$ ),  $\text{NH}_4\text{SCN}$ ,  $\text{HCl}$ ,  $\text{NaOH}$ ,  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  were purchased from Sigma-Aldrich (Egypt). Ethanol 70 % ( $\text{C}_2\text{H}_6\text{O}$ ), Methanol ( $\text{CH}_3\text{OH}$ ), Acetone ( $\text{CH}_3\text{COCH}_3$ ) and Benzene ( $\text{C}_6\text{H}_6$ ) were purchased from Alfa Chemika (Egypt).

Penicillin (standard antibacterial) and fluconazole (standard antifungal) were purchased from Pfizer Co., Ltd. Dimethyl formamide (DMF) was purchased from Sigma, USA.

### Apparatus

For IR spectra in the  $4000\text{-}400 \text{ cm}^{-1}$  range, a JASCO IR-410 spectrometer (Germany) was employed. All absorbance measurements were taken with a JASCO UV/VIS Spectrometer V-630 (Japan). All pH values were taken with a JENWAY 3510 pH-meter (UK). For the weighing procedure, an electronic balance SHIMADZU TW423L (Japan) was employed.

### Methods

#### Preparation and characterization of TPU/Vanillin

**TPU:** A total of ten grams of polyurethane foam (PUF) cubies were immersed in  $\text{HCl}$  (1 mol/L) overnight before being rinsed with distilled water. PUF cubies were washed in 50 mL of concentrated  $\text{HCl}$ , followed by 25 mL of  $\text{NH}_4\text{SCN}$  solution (5 g/L) (Moawed et al., 2022).

**TPU/Vanillin:** Four grams of thiourea polyurethane foam was refluxed in 200 mL

ethanol with two grams vanillin for 2 hours at  $60^\circ\text{C}$ . TPU/Vanillin was washed, dried, and mixed overnight.

**Characterization:** To identify the functional groups, the FTIR spectra of TPU/Vanillin was investigated in the  $400$  to  $4000 \text{ cm}^{-1}$  range. TPU/Vanillin UV/Vis measurements were performed in the  $200\text{-}900 \text{ nm}$  range, and the band gap energy ( $E_g$ ) was calculated using the  $T_{\text{auc}}$  equation;  $(\alpha h\nu)^2 = C (E_g - h\nu)$ ; Where  $h\nu$  (eV) is the energy ( $h\nu = 1240/\lambda$ ),  $\lambda$  (nm) is the wavelength, and  $C$  is a constant.

TPU/Vanillin magnetic susceptibility  $\chi_g$  was estimated using Evans balance data using the equation:  $\chi_g = CL(R - R_0)/10^9(M - M_0)$ . Where  $C$  is constant (1.35 cm),  $L$  is the sample height in cm,  $R$  is the sample in tube balance reading,  $R_0$  is the empty tube balance reading,  $M$  is the mass of the sample in gm, and  $M_0$  is the mass of the empty tube in gm.

The acidic and basic sites of TPU/Vanillin were determined using Boehm's titration. TPU/Vanillin (0.5 gm) was added to  $\text{NaHCO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{NaOH}$  and  $\text{HCl}$  (0.05 mol/L). The solutions were titrated against  $\text{HCl}$  and  $\text{NaOH}$  (0.05 mol/L) after soaking for 24 hours.

The surface charge of TPU/Vanillin was determined at various pH (2-14) and zero-point charge ( $\text{pH}_{\text{PZC}}$ ) levels. After 24 hours, the pH was tested after adding 0.5 gram of TPU/Vanillin to 10 ml of each solution.

TPU/Vanillin's chemical stability was investigated in several buffer solutions (pH: 2-14) and organic solvents such as  $\text{CH}_3\text{OH}$ ,  $\text{CH}_3\text{COCH}_3$ ,  $\text{C}_6\text{H}_6$ ,  $\text{C}_6\text{H}_5\text{CH}_3$ , DMF, and DMSO. After soaking 0.5 g of TPU/Vanillin in 10 mL of each solution and solvent for 24 hours, it was filtrated, dried, and weighted.

#### Antimicrobial activity evaluation

TPU/Vanillin was tested for antimicrobial activity against *B. cereus*, *E. coli*, and *A. niger* using the agar well-diffusion technique according to Balouiri et al. (2016). Nutrient broth, nutrient agar, and Dox agar were prepared and sterilized for 15 minutes at  $121^\circ\text{C}$ . Microbial cultures were adjusted to 0.5 McFarland standards ( $1\text{-}2 \times 10^8 \text{ CFU/mL}$ ) for bacterial strains and  $10^6 \text{ CFU/mL}$  for fungal strain. The microbial culture was then specifically inoculated (100  $\mu\text{l}$ ) into the agar media. In triplicate, the inoculated agar medium was put into sterile Petri dishes. After solidification, a sterile corkborer was used to

make tiny wells (5 mm). Separately, 300 µg/mL of TPU/Vanillin, penicillin (antibacterial), and fluconazole (antifungal) in DMF were added to the wells. Dox agar plates were incubated at 30 °C for 5 days, whereas nutrient agar plates were incubated at 37 °C for 24 hours. Zones of inhibition of TPU/Vanillin were measured in millimeters (mm) ± Standard error (SE) after incubation periods.

#### Minimum inhibition concentration (MIC)

The MIC of TPU/Vanillin against *E. coli*, *B. cereus*, and *A. niger* was investigated (El-Fall et al., 2023). Nutrient broth and Dox broth were made, autoclaved at 121°C for 15 minutes, then cooled at 47°C. In two sets of flasks, 100 µl of *E. coli*, *B. cereus* and *A. niger* spore suspension was inoculated. Each flask was carefully filled with varied levels of the composite (0-1000 µg/ml), with one serving as a positive control to assess microbial cell proliferation in the absence of TPU/Vanillin. A negative control flask containing only cells and DMF was also used. For bacteria or fungi, the flasks were cultured in a shaker incubator (100 rpm) at 37°C for 24 hours or 30°C for 5 days, respectively. The development of microbial growth in the broth was shown by the turbidity of the bacterial broth or pellet formation of fungal growth, and the MIC was measured spectrophotometrically at 600 nm as the lowest concentration of TPU/Vanillin that inhibited the growth of the test bacteria. Fungus biomass was filtered using Whatman filter paper No. 1, dried, and the dry weight (g) calculated.

#### Minimum microbicidal concentration (MBC)

MIC flasks with no apparent growth of microbes were inoculated into nutrient and Dox agar plates and incubated at 37 °C for 24 hours or 30°C for 5 days for bacteria or fungus, respectively. The MBC values of the antimicrobial agents were determined in the absence of visible microbial growth plates.

#### Statistical analysis

The ANOVA test was used to analyze the data using SPSS software version 18. The significance level was set at 0.05. The trials were repeated three times. All findings were presented using the mean and standard error (SE).

## Results

### Characterization of TPU/Vanillin

TPU/Vanillin IR spectra was obtained between 400 and 4000 cm<sup>-1</sup> (Figure 1) to determine its primary functional groups. TPU/Vanillin absorption peaks were detected at 3745-3000, 2944, 2852, 2231, 1633, and 1099 cm<sup>-1</sup>. At 3745-3000 cm<sup>-1</sup>, a wideband of -NH and -OH groups were found. There were many peaks recorded at 2944 cm<sup>-1</sup>, 2852 cm<sup>-1</sup> (-CH), 2231 cm<sup>-1</sup> (N=C=S), 1633 cm<sup>-1</sup> (C=C), and 1099cm<sup>-1</sup> (C-O).

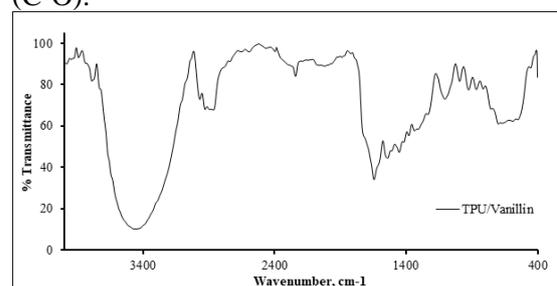


Figure 1. IR spectra of TPU/Vanillin

The Nujol mulls technique was used to determine the UV-Vis absorption spectra of TPU/Vanillin (Figure 2). TPU/Vanillin specific absorption peaks were found at 203, 212, 218, 227, 229, 233, 237, 333, 340, and 370 nm.

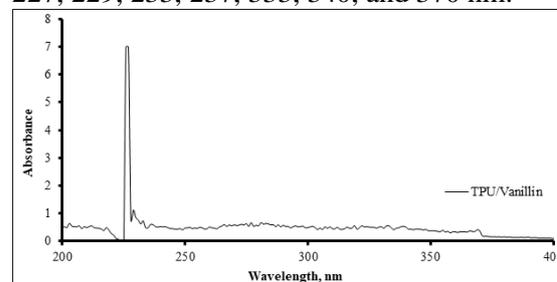
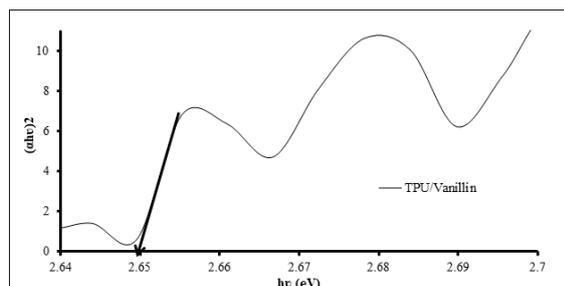


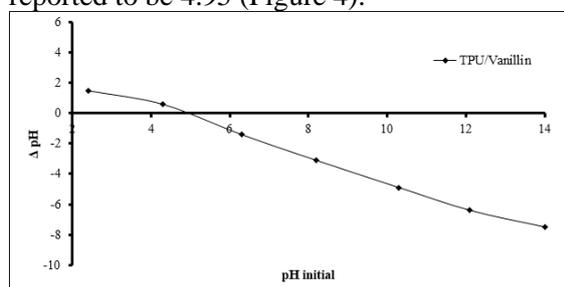
Figure 2. UV-Vis spectrum of TPU/Vanillin

TPU/Vanillin bandgap energy ( $E_g$ ) was calculated from UV-Vis measurements using the  $T_{auc}$  equation;  $(\alpha h\nu)^2 = C(E_g - h\nu)$ . The  $(\alpha h\nu)^2$  were plotted versus  $h\nu$ , and the energy gap at  $h\nu = 0$  may be approximated by the straight section of the  $h\nu$  axis. TPU/Vanillin bandgap energy was calculated to be 2.65 eV (Figure 3).



**Figure 3.** Bandgap energy of TPU/Vanillin

The surface charge of TPU/Vanillin at different pH levels and at zero charge point ( $\text{pH}_{\text{PZC}}$ ) was investigated. The pH discrepancies between the initial and final pH values were plotted against the  $\text{pH}_i$ . The  $\text{pH}_{\text{PZC}}$  of TPU/Vanillin was reported to be 4.95 (Figure 4).



**Figure 4.**  $\text{pH}_{\text{PZC}}$  of TPU/Vanillin

Magnetic susceptibility is a physical characteristic commonly used to characterize para and dia magnetic materials. The magnetic susceptibility  $\chi_g$  of TPU/Vanillin was estimated using the Evans balance data. TPU/Vanillin's magnetic susceptibility was  $-0.25 \times 10^{-6} \text{ cm}^3/\text{mol}$ . TPU/Vanillin was regarded a diamagnetic material with a negative sign of magnetic susceptibility (Table 1).

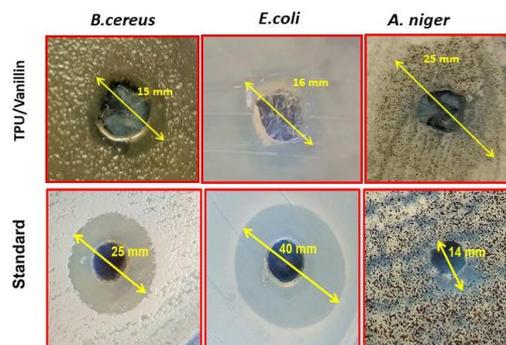
**Table 1.** Magnetic susceptibility of PCPU and PCPU/ZnO

Sorbent	C	L	$R_s$	$R_0$	$M_s$	$M_0$	$\chi_g$
TPU/Vanillin	1.35	1.5	-28	-27	0.7577	0.7496	$-0.25 \times 10^{-6}$

TPU/Vanillin's chemical stability was investigated in several buffer solutions (pH: 2-14) and organic solvents (e.g.,  $\text{CH}_3\text{OH}$ ,  $\text{CH}_3\text{COCH}_3$ ,  $\text{C}_6\text{H}_6$ ,  $\text{C}_6\text{H}_5\text{CH}_3$ , DMF, and DMSO). TPU/Vanillin weights were unaltered, confirming its chemical stability.

#### Antimicrobial activity evaluation

TPU/Vanillin antibacterial activity was tested using the agar well diffusion test against Gram-negative bacteria *E. coli*, Gram-positive bacteria *B. cereus*, and fungus *A. niger* (Figure 5).



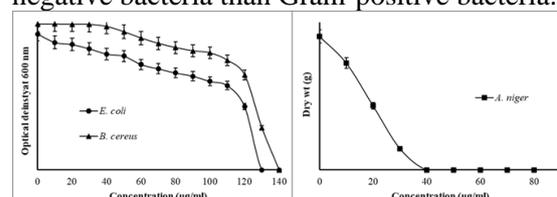
**Figure 5.** Antimicrobial activity of TPU/Vanillin in comparison with penicillin (standard antibacterial) and fluconazole (standard antifungal) using agar well diffusion method against *B. cereus*, *E. coli*, and *A. niger*. Arrows denote the diameter of inhibition zones (mm).

TPU/Vanillin shown moderate antibacterial action against Gram-negative *E. coli* and Gram-positive *B. cereus*. TPU/vanillin particularly inhibited Gram-negative *E. coli* better than Gram-positive *B. cereus*, with inhibition zones of  $16 \pm 0.12$  and  $15 \pm 0.19$  mm, respectively. Penicillin was more effective against bacteria, with inhibition zones of  $40 \pm 0$  mm against *E. coli* and  $25 \pm 0.03$  mm against *B. cereus*. On the other hand, TPU/Vanillin revealed stronger antifungal action against *A. niger* compared to fluconazole (Table 2).

**Table 2.** Zones of inhibition (ZOI) of TPU/Vanillin in comparison with penicillin (standard antibacterial) and fluconazole (standard antifungal)

Compound	Zones of inhibition (mm $\pm$ SE)		
	<i>E. coli</i>	<i>B. cereus</i>	<i>A. niger</i>
TPU/Vanillin	$16 \pm 0.12$	$15 \pm 0.19$	$25 \pm 0.20$
Penicillin	$40 \pm 0$	$25 \pm 0.03$	-
Fluconazole	-	-	$14 \pm 0.14$

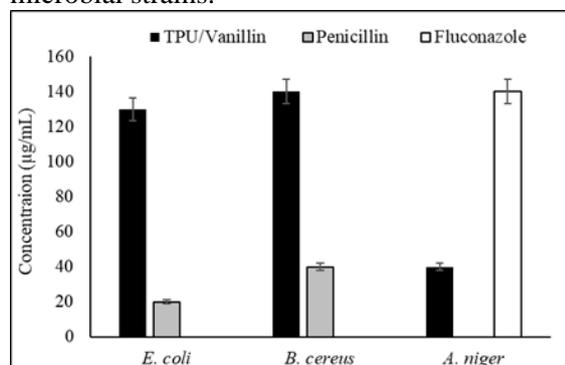
As shown in Figure 6, TPU/Vanillin had MIC values of 130, 140, and 40  $\mu\text{g}/\text{ml}$  against *E. coli*, *B. cereus*, and *A. niger*. These findings revealed that TPU/Vanillin exhibited more antifungal activity than antibacterial activity, as well as greater antibacterial activity against Gram-negative bacteria than Gram-positive bacteria.



**Figure 6.** MIC of TPU/Vanillin against *E. coli*, *B. cereus* and *A. niger*.

Figure 7 depicts the MBC results of TPU/Vanillin against *E. coli*, *B. cereus*, and *A. niger*. TPU/Vanillin outperformed fluconazole

in terms of fungicidal activity against *A. niger*. When compared to the standard drugs, TPU/Vanillin displayed moderate to high antimicrobial activity against the tested microbial strains.



**Figure 7.** MMC of TPU/Vanillin against *E. coli*, *B. cereus* and *A. niger*.

## Discussion

Microbial resistance to antimicrobials in common usage is becoming increasingly common. Further debate is framed by a historical viewpoint. Although bacterial resistance is the most prevalent, resistance has been found in fungi, viruses, and parasites. Resistance is a multifaceted phenomenon that includes the microbe, the environment, and the patient all at once. Resistance may be inherent in the microorganism prior to medication exposure or may develop because of therapy (Cohen et al., 1997). Consequently, unique, and emerging polymer-based antimicrobial chemotherapeutic materials have gained a lot of interest. Antibacterial and antifungal effects of thiourea and vanillin have been thoroughly explored (Rizwana, & Lakshmi, 2012; Alwan, 2018). They have antimicrobial properties due to their harmful effects on the microbial cell permeability moreover the formation of reactive oxygen species (ROS) (Fitzgerald et al., 2004; Roman et al., 2023). The present study aimed to prepare thiourea polyurethane composite modified with vanillin and explore its antimicrobial properties.

Infrared spectra, Ultraviolet-Visible spectra, bandgap energy, X-ray diffraction, magnetic susceptibility, and  $pH_{PZC}$  were used to characterize TPU/vanillin composites. IR spectrum of TPU/Vanillin confirmed the presence of  $-NH$ ,  $-OH$ ,  $-CH$ ,  $N=C=S$ ,  $C=C$  and  $C-O$  at the absorption peaks at 3745-3000, 2944, 2852, 2231, 1633, 1099,  $cm^{-1}$ . The pH of

zero point charge ( $pH_{PZC}$ ) measurement was helped in predicting the surface behavior of composite (Boumediene et al., 2018). The surface charge of TPU/Vanillin at various pH levels and at zero charge point ( $pH_{PZC}$ ) was investigated. TPU/Vanillin surfaces will be positively charged at pH 4.95 and negatively charged at pH > 4.95. Boehm titration was used to characterize carboxylic, lactonic and phenolic hydroxyl groups. Base solutions such as sodium hydroxide (NaOH) neutralize all three functionalities, sodium carbonate ( $Na_2CO_3$ ) neutralize carboxylic and phenolic groups, and sodium bicarbonate ( $NaHCO_3$ ) only neutralize phenolic groups (Kim et al., 2011; Schönherr et al., 2018). The acidic sites of TPU/Vanillin were 49 mmol/g while basic sites were negligible. Magnetic susceptibility confirmed the negative susceptibility of TPU/Vanillin as a diamagnetic material. TPU/Vanillin chemical stability test confirm its chemical stability in different buffer solutions (pH: 2–14) and organic solvents including  $CH_3OH$ ,  $CH_3COCH_3$ ,  $C_6H_6$ ,  $C_6H_5CH_3$ , DMF, and DMSO).

Agar well diffusion method was used to calculate the zone of inhibition of TPU/Vanillin against *E. coli*, *B. cereus* and *A. niger*.

TPU/Vanillin revealed a moderate antibacterial activity against *E. coli* and *B. cereus* while it showed potent antifungal action against *A. niger* with inhibition zones of  $16 \pm 0.12$ ,  $15 \pm 0.19$  and  $25 \pm 0.20$  mm, respectively. Also, Rizwana, & Lakshmi (2012) reported the bactericidal activity of vanillin and N-allyl thiourea against *Klebsiella pneumoniae*, *B. cereus* and *Pseudomonas aeruginosa* and its fungicidal activity against *A. niger*, *Candida albicans* and *Candida kefyr*. It produced inhibition zones of 23 and 36 mm against *B. cereus* and *A. niger*, respectively. Similarly, Buslovich et al. (2017) reported the antimicrobial action of vanillin/polyethylene composite against Gram-negative *E. coli* was higher than and Gram-positive *Staphylococcus aureus* bacteria.

The MMC is the smallest amount of an antimicrobial agent required to eliminate a certain microbe over a lengthy period of time, such as 18 or 24 hours. Antimicrobial agents are considered microbicidal if the MMC is less than four times the MIC. The MMC results of TPU/Vanillin matched with the MIC values against *E. coli* and *B. cereus* and *A. niger* (130, 140 and 40  $\mu g/ml$ ) demonstrating its moderate

to strong biocidal activity. While Patel et al. (2017) documented the antimicrobial activity of some thiourea derivatives against *E. coli*, *S. aureus* and *C. albicans* with MIC values of 40-160, 80-160, 80-320 µg/ml, respectively.

TPU/Vanillin had a higher antibacterial activity against *E. coli* than *B. cereus*. Gram-positive bacteria (*B. cereus*) have thicker peptidoglycan layers than Gram-negative bacteria (*E. coli*), which increases the negative charge value of the cell wall (Schleifer & Kandler, 1972). Gram-positive bacteria have greater negative charge values in their cell walls due to bigger peptidoglycan coats. Gram-positive bacteria repel Gram-negative bacteria more strongly, which may limit their antibacterial action (Isticato & Ricca, 2016).

The surface charging behavior of TPU/Vanillin was used to analyze the mechanism of antibacterial action. Bacterial cell walls contain a negative charge because acidic substances like peptidoglycan are present (Schleifer & Kandler, 1972). As the characterization findings were accepted, the surface charge of TPU/Vanillin was mostly acidic. TPU/Vanillin's negative charge may cause repulsion between it and bacterial cells, reducing its antibacterial activity as compared to the usual antibacterial drug, penicillin.

Vanillin demonstrated potent antibacterial activity against Gram-positive and Gram-negative microorganisms by inhibiting growth, viability, and biofilm formation. The antibacterial properties were achieved by incorporating biologically active components in vanilla as an alternative or adjunct antibiotic option to treat illnesses caused by a variety of bacterial diseases (Maisch et al., 2022). Many thiourea compounds also shown antibacterial activity against *B. cereus* and *E. coli* (Marzi et al., 2019). Furthermore, TPU/Vanillin shown greater antifungal activity against *A. niger* ( $25 \pm 0.20$  mm, MIC & MMC; 40 µg/ml) than fluconazole ( $14 \pm 0.14$  mm, MIC & MMC; 140 µg/ml). TPU/Vanillin suppress spore germination and deactivate spores, which is necessary to minimize fungal infection and mycotoxin generation. The rupture of cell walls and membranes, which results in microbial morphological alterations, is the fundamental mechanism underpinning TPU/Vanillin's antifungal actions (Li et al., 2021). Vanillin's antifungal actions were related to the destruction of the integrity of cell membranes rather than cell walls (Li et al., 2014). The

aldehyde moiety is important in vanillin's antifungal action, although the side-group location on the benzene ring also regulates its activity (Fitzgerald et al., 2005).

## Conclusion

Immobilization of vanillin within thiourea polyurethane foam resulted in vanillin thiourea polyurethane foam (TPU/Vanillin). IR, UV/Vis, bandgap energy, pHPZC, magnetic susceptibility, and chemical stability were used to identify TPU/Vanillin. TPU/Vanillin was tested for antibacterial action against *E. coli*, *B. cereus*, and *A. niger* in vitro. TPU/vanillin was more effective against gram-negative bacteria than gram-positive bacteria. TPU and vanilla prevent spore germination and deactivate fungus spores. TPU/Vanillin has been shown to be an effective antibacterial agent by successfully reducing the development of microbiological strains.

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## الملخص العربي

### عنوان البحث: تحضير وتوصيف مركب البولي يوريثان المعدل بالفانيلين المضاد للميكروبات

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بعد تعديل مركبات البولي يوريثان بواسطة الفانيلين والثيوريا بمثابة بحث عن مركب نشط جديد مضاد للميكروبات. تم تحضير وتقييم مركبات البولي يوريثان المحملة بالفانيلين والثيوريا (البولي يوريثان ثيوريا / الفانيلين) لنشاطها المضاد للميكروبات. مركب البولي يوريثان ثيوريا / الفانيلين يجمع بين الخصائص السطحية لرغوي البولي يوريثان والوظائف البيولوجية للثيوريا والفانيلين. تم التعرف على مركب البولي يوريثان ثيوريا / الفانيلين بواسطة الأشعة تحت الحمراء والأشعة فوق البنفسجية وطاقة فجوة النطاق والقابلية المغناطيسية والثبات الكيميائي ودرجة الحموضة لشحنة نقطة الصفر. تم مركب البولي يوريثان ثيوريا / الفانيلين للتأكد من فعاليتها المضادة للبكتيريا والفطريات. تمت دراسة النشاط المضاد للميكروبات في المختبر لمركب البولي يوريثان ثيوريا / الفانيلين

ضد البكتيريا سالبة الجرام (إيشريشيا كولاي) و بكتيريا إيجابية الجرام (باسيليس سيريس) والفطريات (أسبرجيلس نيجر). تم استخدام طريقة انتشار المواد خلال الأجار معمليا لحساب منطقة التثبيط لمركب البولوي يوريثان ثيوريا / الفانيلين ضد إيشريشيا كولاي ( $0.12 \pm 0.16$  مم) و باسيليس سيريس ( $0.15 \pm 0.19$  مم) و أسبرجيلس نيجر ( $0.20 \pm 0.25$  مم). أثبت تثبيط نمو السلالات الميكروبية بنجاح أنه يمكن تحسين البولوي يوريثان ثيوريا / الفانيلين بشكل أكبر للتطبيقات المحتملة مثل تغليف المواد الغذائية والطلاء. أثبت اختبار تثبيط نمو السلالات الميكروبية نجاحا كبيرا وذلك عن طريق استخدام قيم تركيز من مركب البولوي يوريثان ثيوريا / الفانيلين تبلغ 130 و 140 و 40 ميكروجرام/مل ضد إيشريشيا كولاي، باسيليس سيريس و أسبرجيلس نيجر، على التوالي، إن مركب البولوي يوريثان ثيوريا / الفانيلين الواعد يمكن تحسينه واستخدامه بشكل كبير في التطبيقات المختلفة مثل تغليف المواد الغذائية والطلاء.