

Clinical Applications of Schiff Base Nanocomposites with ZnO and TiO₂ Nanoparticles: Evaluation of their Enzymatic and Biological Activities for Pathological Diagnostics

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ABSTRACT

Objective: This study aimed to synthesize and characterize Schiff base nanocomposites using ZnO and TiO₂ nanoparticles for potential clinical applications in diagnosing and treating infectious diseases and liver function disorders. **Methods:** Schiff bases (SA1-SA5) were synthesized from 4-aminoantipyrine and various carboxaldehydes. Characterization was performed using melting point analysis, Fourier-transform infrared spectroscopy (FTIR), and proton nuclear magnetic resonance (HNMR). Nanocomposites were then prepared by doping Schiff bases with 20% ZnO and TiO₂ nanoparticles, utilizing ultrasound irradiation for enhanced integration. X-ray diffraction (XRD) and field emission scanning electron microscopy (FESEM) were employed to analyze the nanocrystal structures and morphologies. **Results:** The synthesized nanocomposites demonstrated significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and antifungal activity against *Candida albicans*, indicating their potential for treating infections. Additionally, the compounds were evaluated for their effects on alkaline phosphatase activity in models of liver dysfunction. The compounds showed promising results in modulating enzyme activity, suggesting potential applications in diagnosing and monitoring liver diseases. **Conclusion:** The Schiff base nanocomposites prepared with ZnO and TiO₂ nanoparticles show significant potential in pathological diagnostics and as therapeutic agents. Their ability to inhibit microbial growth and modulate enzyme activity in liver disease models highlights their utility in clinical pathology, warranting further investigation for medical applications.

Keywords: 4-Aminoantipyrine, Schiff Base, Zinc Oxide Nanocomposites, Titanium Dioxide Nanocomposites, Alkaline Phosphatase Enzyme

INTRODUCTION

The continuous pursuit of innovation in laboratory diagnostics and clinical pathology has led to an increased interest in integrating nanotechnology with biomedical sciences. Nanomaterials, due to their unique physicochemical properties and high surface-to-volume ratios, are revolutionizing traditional laboratory practices by enhancing diagnostic sensitivity, antimicrobial efficacy, and enzyme modulation capabilities[1]. Among these materials, Schiff base compounds and their nanocomposites, particularly those combined with metal oxide nanoparticles such as zinc oxide (ZnO) and titanium dioxide (TiO₂)[2], have emerged as promising tools with multifaceted applications in medical diagnostics, microbial control, and biochemical analysis. Schiff bases are nitrogen-containing compounds formed through the condensation of primary amines with aldehydes or ketones, resulting in imine (C=N) functional groups[3]. These compounds are widely recognized for their chelating ability, thermal stability, and versatile biological activity. In the field of laboratory medicine, Schiff bases have garnered attention for their antimicrobial, antioxidant, anti-inflammatory, and enzyme inhibitory properties. Structurally tunable, Schiff base derivatives can be synthesized with specific functional groups to target microbial membranes or interact with enzymatic active sites, making them suitable for use in diagnostic assays and therapeutic formulations[4]. The integration of metal oxide nanoparticles such as ZnO and TiO₂ with Schiff bases further enhances their biological potential. Zinc oxide nanoparticles possess well-documented antimicrobial, antifungal, and anti-inflammatory activities. Their mechanism of action involves the generation of reactive oxygen species (ROS), disruption of microbial membranes, and inhibition of microbial enzyme function[5]. ZnO NPs are also known for their

biocompatibility, making them ideal candidates for use in biomedical applications such as wound healing, biosensor development, and drug delivery systems. Similarly, titanium dioxide nanoparticles have been widely explored for their photocatalytic behavior, biocompatibility, and their role in sterilization and disinfection processes[6]. TiO₂ is effective against a broad spectrum of microbial pathogens and has shown potential in surface coatings for medical devices and laboratory instruments to reduce nosocomial infections. When incorporated into Schiff base frameworks, these nanoparticles form nanocomposites that exhibit synergistic biological effects [7-11]. These Schiff base-metal oxide nanocomposites can be tailored to target specific biological functions or diagnostic markers. Of particular relevance to laboratory medicine is their ability to inhibit the growth of clinically significant pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. These organisms are frequently encountered in clinical microbiology laboratories and are major contributors to hospital-acquired infections. By evaluating the antimicrobial efficacy of these nanocomposites against such pathogens, this study contributes to the growing need for novel, non-antibiotic antimicrobial agents to support infection control and diagnostic accuracy[12-16]. Another critical area of interest is the interaction of these nanocomposites with biochemical markers, particularly liver function enzymes such as alkaline phosphatase (ALP). ALP is a routinely used marker in clinical laboratories for diagnosing hepatobiliary diseases, bone disorders, and systemic infections. Modulation of ALP activity by bioactive compounds suggests potential applications not only in therapy but also in biochemical assay development. Compounds that influence ALP activity may provide diagnostic insight into disease progression or response to treatment,

supporting the integration of novel nanomaterials into existing laboratory protocols. The present study is designed to address the intersection between nanomaterial innovation and diagnostic pathology.

Schiff base compounds were synthesized using 4-aminoantipyrine and various aromatic aldehydes, followed by doping with ZnO and TiO₂ nanoparticles to generate nanocomposites with enhanced bioactivity. These materials were subjected to comprehensive physicochemical characterization using Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (¹H NMR), X-ray diffraction (XRD), and field emission scanning electron microscopy (FESEM) to confirm their structural integrity and nanoscale morphology [17-20]. The biological performance of the synthesized nanocomposites was evaluated through in vitro antimicrobial testing and enzyme interaction assays to assess their clinical utility in pathological diagnostics. By combining organic synthesis with nanotechnology and laboratory-based analysis, this study aims to bridge the gap between chemical innovation and clinical

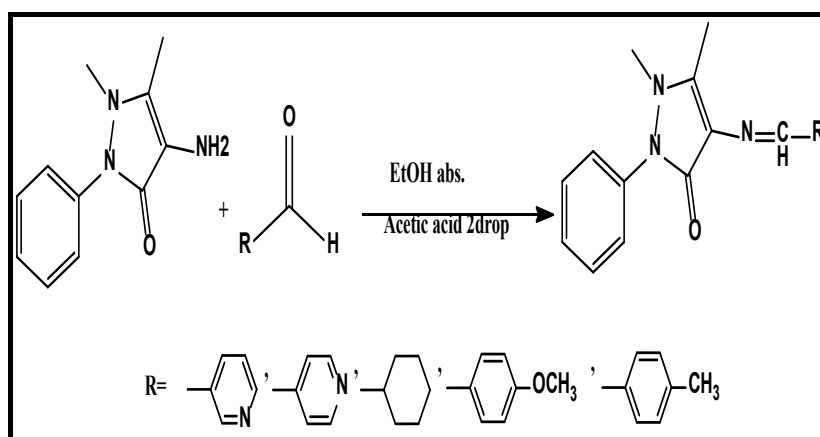
application. The findings may contribute to the development of next-generation diagnostic agents capable of dual action: detecting disease biomarkers and simultaneously managing infections, a paradigm increasingly important in modern laboratory medicine, particularly in regions with limited access to advanced therapeutic resources.

EXPERIMENTAL PART

Synthesis the Compounds

Synthesis of the Compounds (SA₁-SA₅) from the 4-aminoantipyrine

Schiff bases were synthesized by the condensation reaction of 4-aminoantipyrine (0.005 mol), with different aldehydes (cyclohexane carboxaldehyde, 4-methoxy carboxaldehyde, 3-methyl carboxaldehyde, 4-pyridine carboxaldehyde, 3-pyridine carboxaldehyde) (0.005) mol Added to it catalytic Glacial Acetic Acid (2 drops) and stirred and refluxed for 6 hours in ethanol absolute (10 ml). It was filtrated and washed with water and ethanol and recrystallized in ethanol to give compound (SA₃), Table (1), [21].



Scheme A: Synthesis of Compounds (SA₁-SA₅)

Table 1: Physical Properties Data of Prepared Compounds

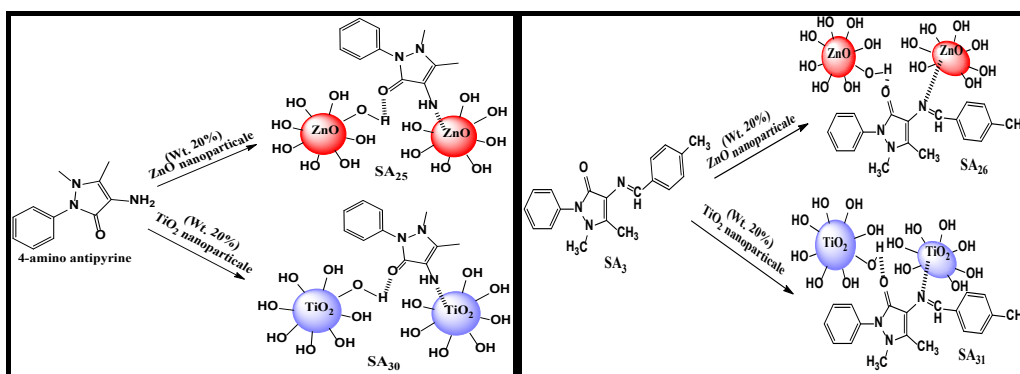
Comp. No	R	Molecular Formula	Color	M.P(°C)	Yield %
SA ₁		C ₇ H ₁₁	Pale Yellow	149-151	73
SA ₂		C ₈ H ₇ O	Yellow	167-168	87
SA ₃		C ₈ H ₇	Yellow	184-186	86
SA ₄		C ₆ H ₄ N	Yellow	203-205	84
SA ₅		C ₆ H ₄ N	Yellow	239-241	88

Synthesis of the Nanocomposites

Nanocomposites were synthesized by the dried (0.003 mol) (4-aminoantipyrine) (SA₃) was added into absolute dioxane (10mL) with the addition of zinc oxide nanoparticles and titanium oxide nanoparticles (Wt. 20%) of each, dispersed the mix ultrasound for 4

hours to prepare nanocomposites (SA₂₅) and (SA₂₆) and prepare nanocomposites (SA₂₉) and (SA₃₀) then they were stirring at a temperature 60 °C, for a period 4 hours of then.

The product was collected and washed three times using deionized water. Dry the residue at 80 °C until the weight is stable [16].

**Scheme B:** Synthesis of Compounds (SA₂₅, SA₂₆)**Scheme C:** Synthesis of Compounds (SA₃₀, SA₃₁)**Collection Sites and Dates:**

While the synthesis of chemical compounds does not directly involve field collection, the development of the taxonomic key for newly recorded genera (if applicable) would necessitate detailed records of collection sites and dates. This would involve specifying the geographic locations and environmental conditions under which samples or data were collected, including the use of molecular techniques for genus identification.

Source and Justification for Taxonomic Keys

The taxonomic key for newly recorded genera (if applicable) was developed based on a comprehensive review of existing literature, databases, and consultations with experts in the field. This process included identifying gaps in current taxonomic keys and making

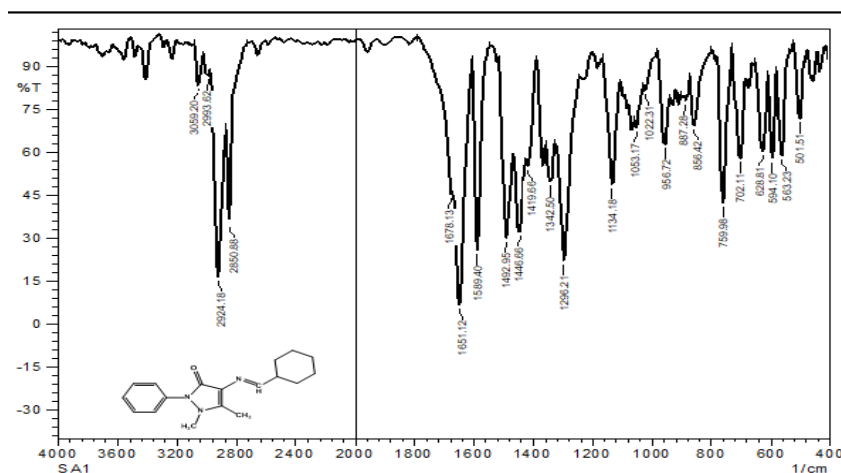
necessary adaptations to include the new genera. The justification for these adaptations was rooted in the need to accurately reflect the unique characteristics of the newly identified genera, ensuring that the taxonomic key serves as a valuable tool for researchers and practitioners in the field.

Results and Discussion**FTIR spectrum**

The FTIR spectra of compounds (SA₁- SA₅) showed absorption at (3059-3036) cm⁻¹ for (C-H), aromatic asymmetrical and symmetrical stretching, and (C-H) aliphatic at (2997-2924) cm⁻¹, strong stretching absorption at about (1697-1666) cm⁻¹ for (C=O) and (1651-1643) cm⁻¹ for (C=N). These are presented in Table (2) and Figures (1, 2, 3, 4, and 5) [22].

Table 2: Characteristic Bands of FT-IR Spectra ν (cm⁻¹)

Comp. No.	C-H Arom.	C-H Aliph. Asy Sy	C=O amide	C=N	C=C
SA ₁	3059	2993 2924	1678	1651	1589
SA ₂	3051	2993 2924	1666	1647	1589
SA ₃	3055	2997 2928	1678	1651	1585
SA ₄	3043	2997 2951	1670	1643	1589
SA ₅	3036	2993 2951	1697	1643	1597

**Figure 1:** FT-IR Spectrum for Compound (SA₁)

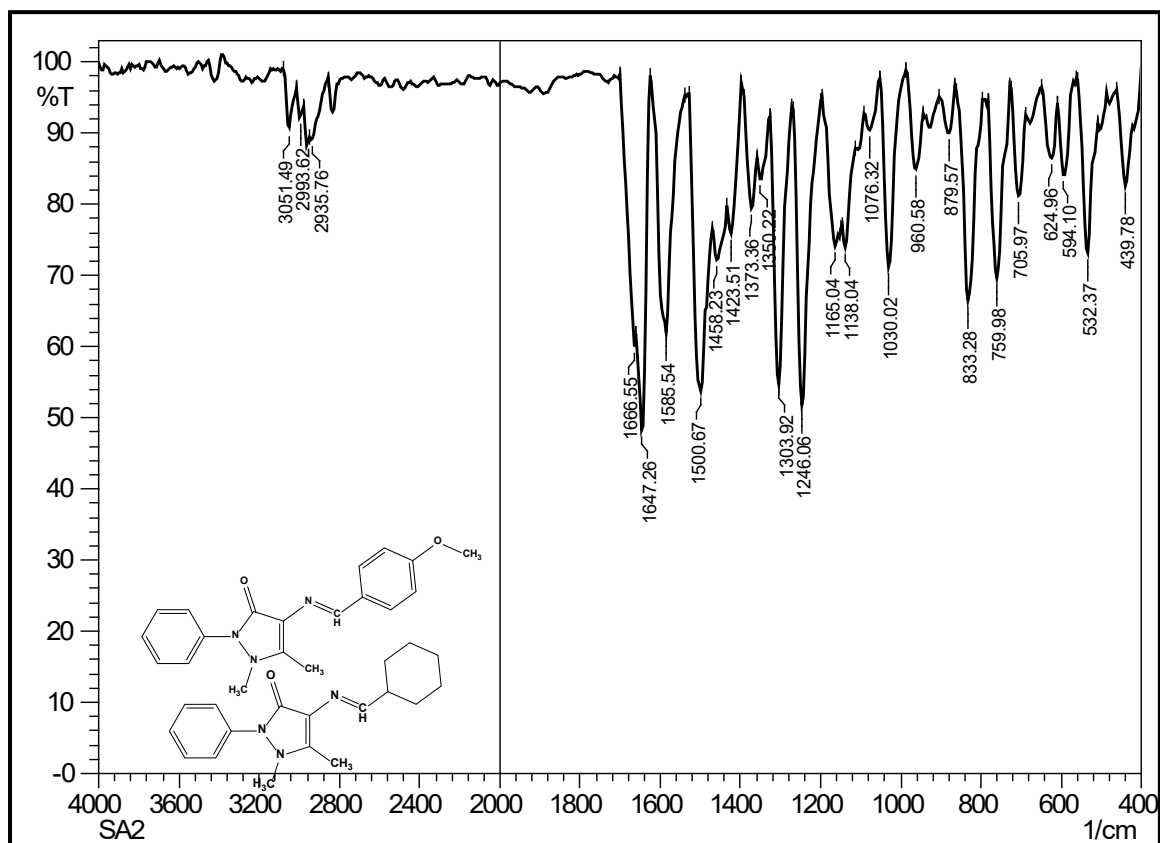


FIGURE 2: FT-IR Spectrum for Compound (SA₂)

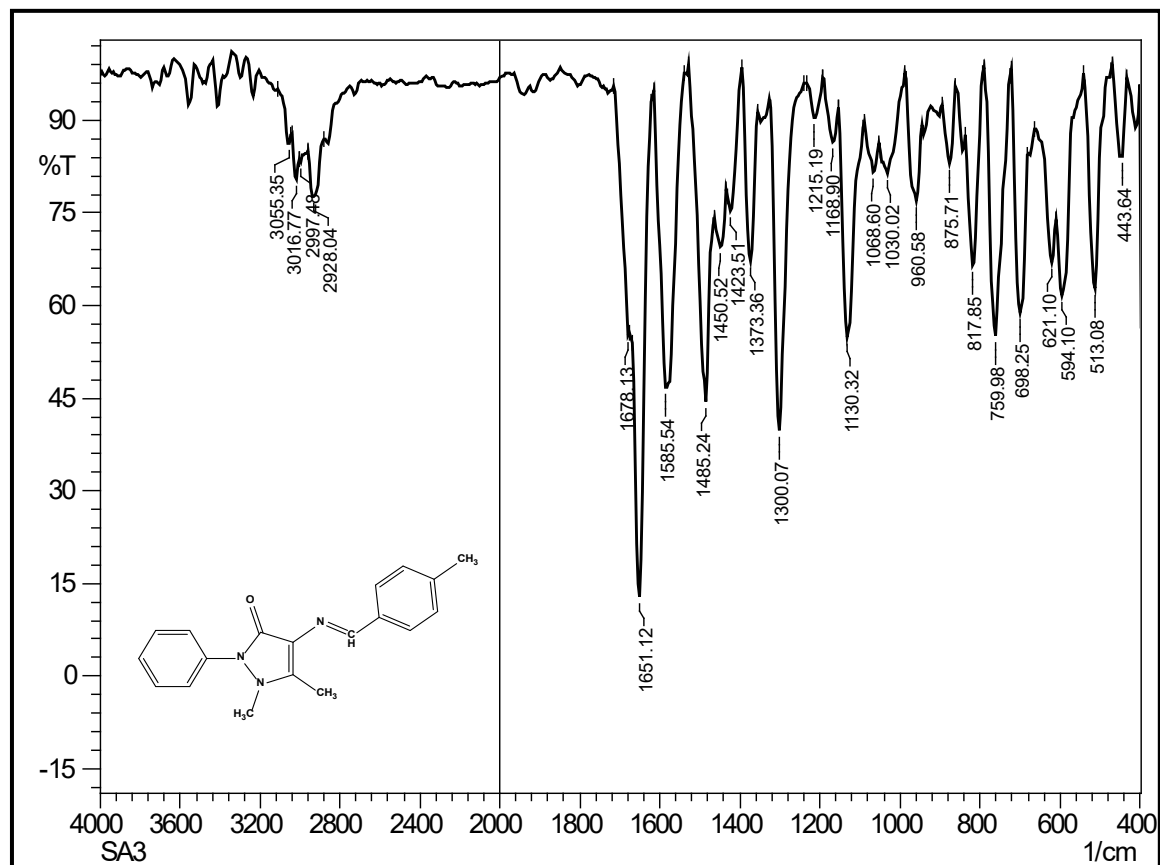


FIGURE 3: FT-IR Spectrum for Compound (SA₃)

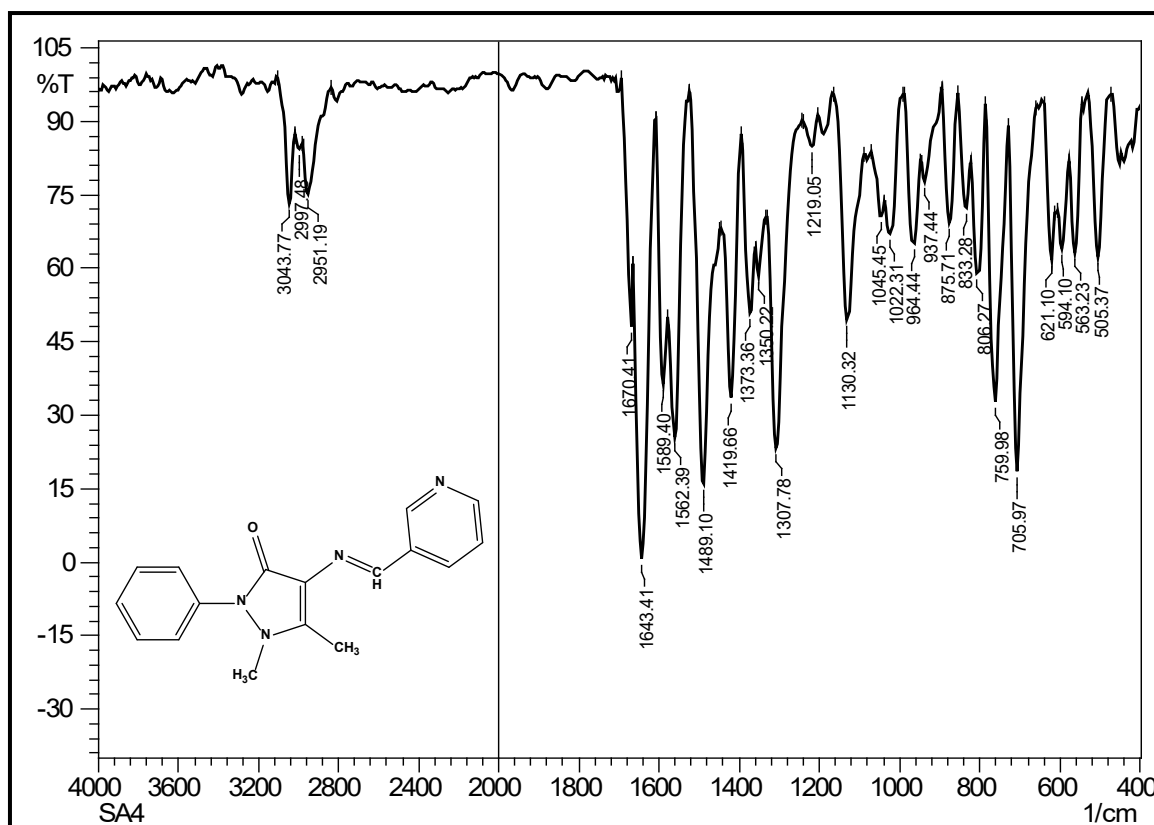


Figure 4: FT-IR Spectrum for Compound (SA₄)

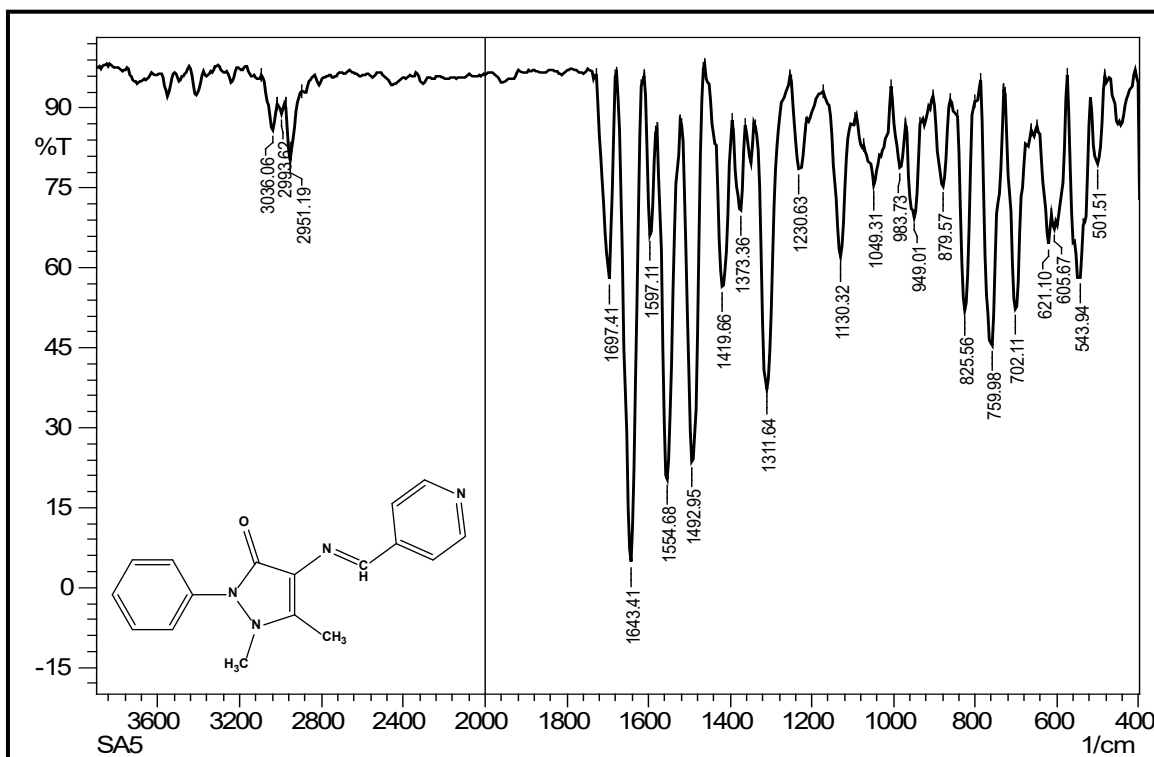
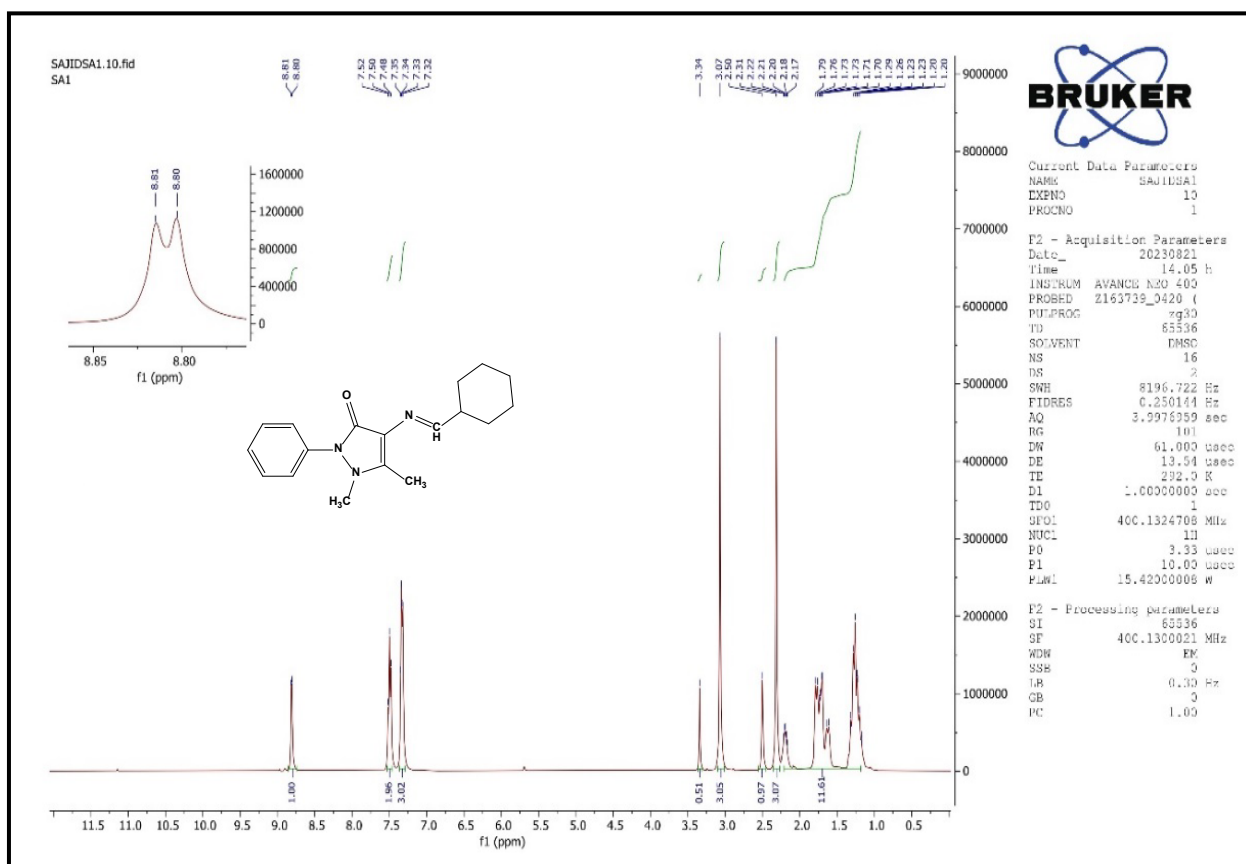
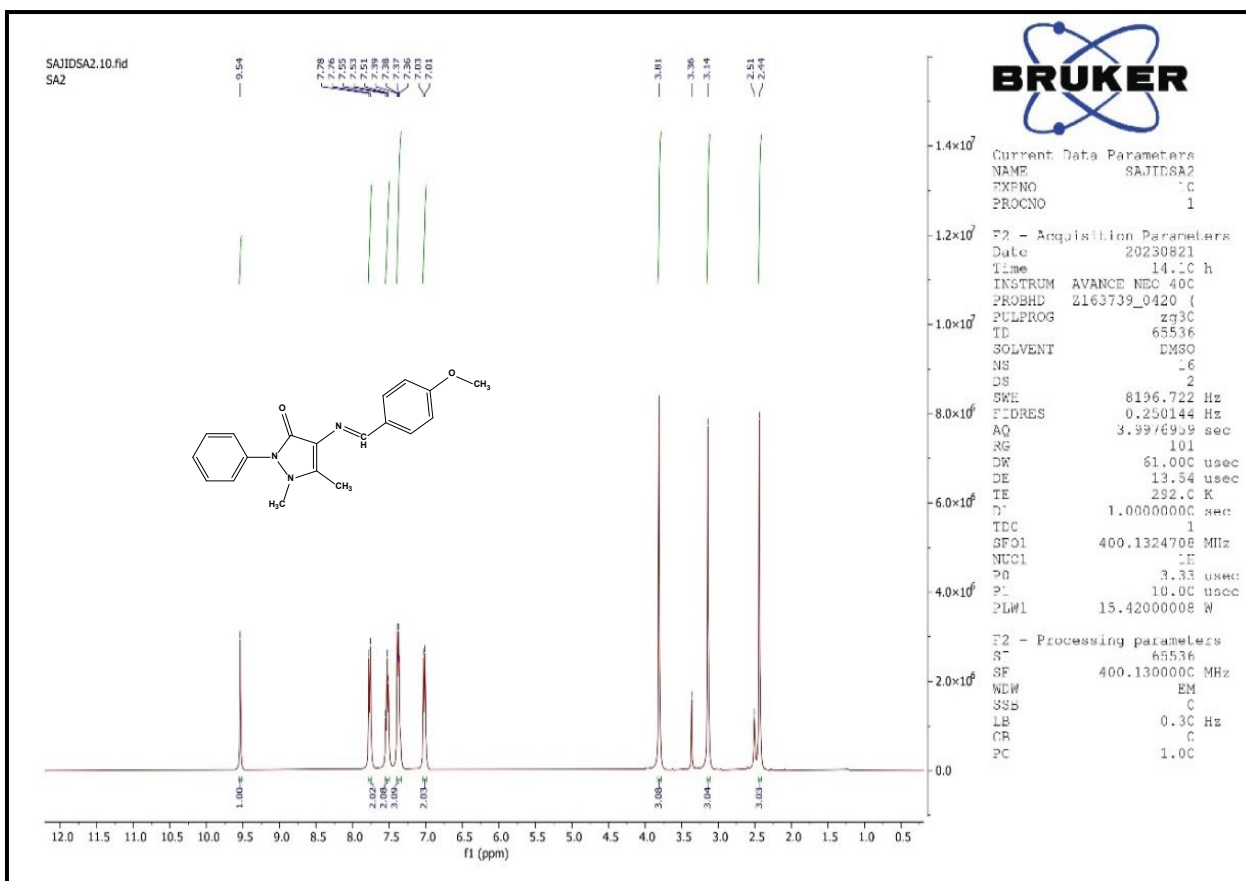


Figure 5: FT-IR Spectrum for Compound (SA₅)

NMR Spectrum

The NMR spectra of compounds (SA₁- SA₅) were recorded in dimethyl sulfoxide (DMSO). The chemical shifts of the different types of protons and carbons are given in the experimental part. Where showed signals at (8.81-9.63 δ) back to (CH) Schiff bond and in (SA₄,

SA₅) showed signals at (8.94-8.21 δ) back to (C-N) Schiff bond in pyridine ring. It showed signals of multi-at (7.78-7.01 δ) back to protons aromatic, singles sign (3.81-1.2 δ) back to protons (CH₃), and the sign at (2.51 δ) back to the (DMSO) protons. Figure (6, 7, 8, 8, 9, 10), [22].

Figure 6: ¹H.NMR Spectrum for Compound (SA₁)Figure 7: ¹H.NMR Spectrum for Compound (SA₂)

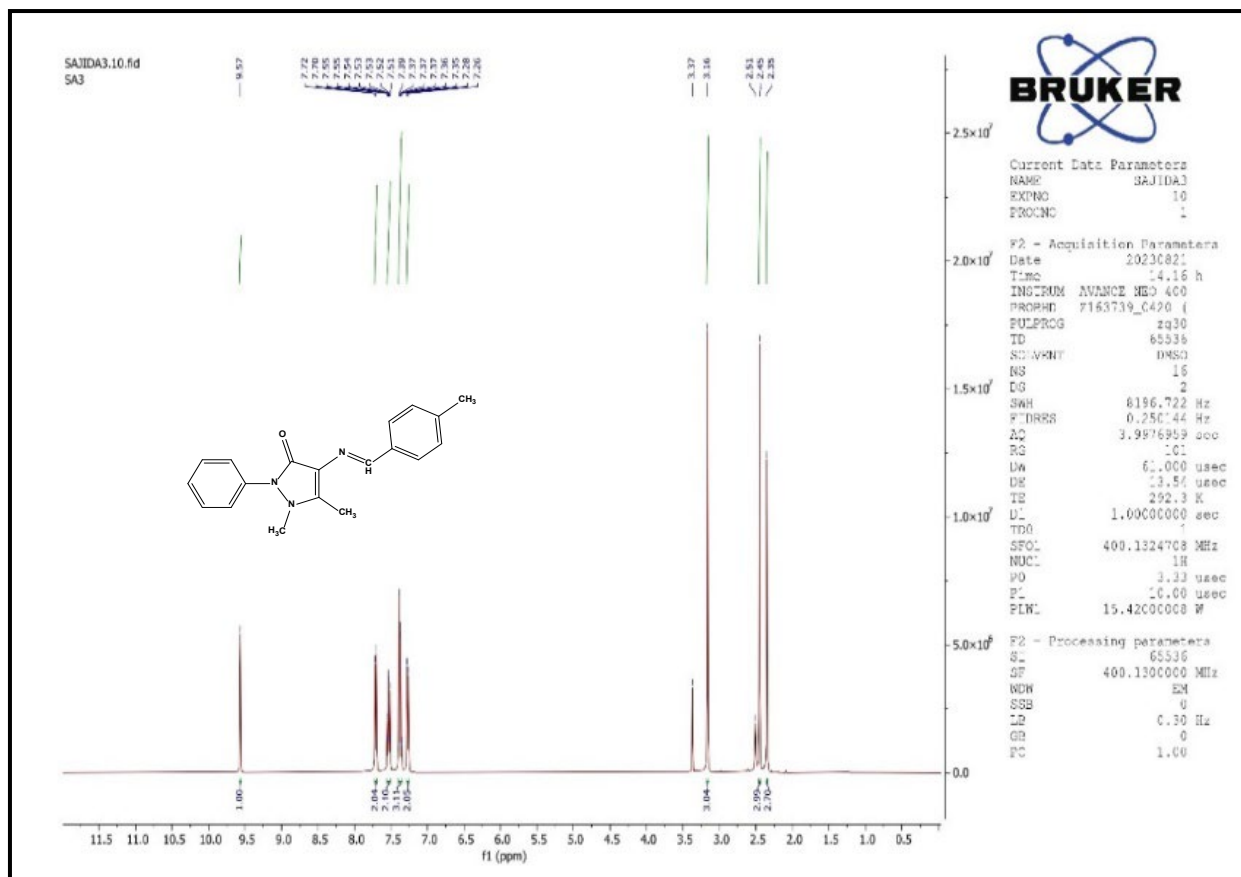


Figure 8: ¹H-NMR Spectrum for Compound (SA₃)

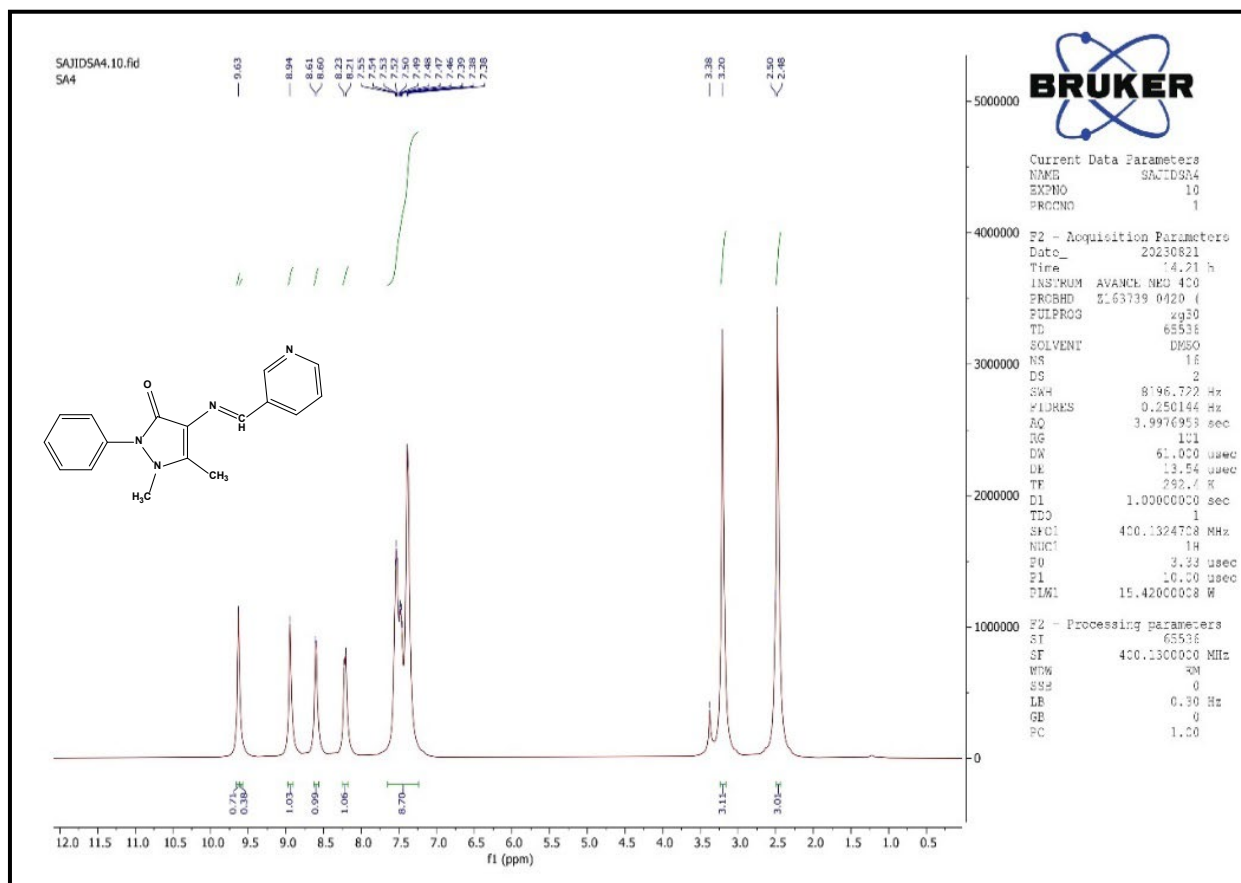


Figure 9: ¹H-NMR Spectrum for Compound (SA₄)

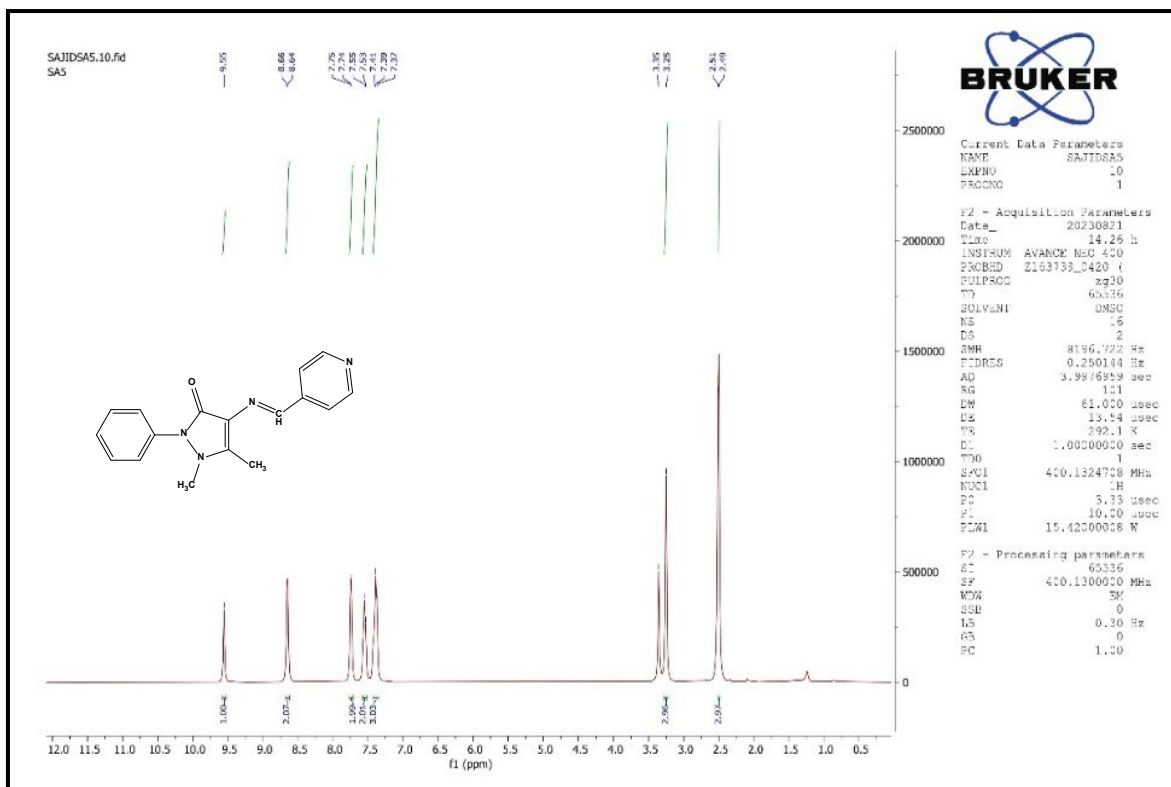


Figure 10: ^1H -NMR Spectrum for Compound (SA₅)

X-Ray Diffraction (XRD) Analysis

1- Nanocomposites: SA₂₅: To assess the purity and crystallinity of the ZnO thin films, XRD data were obtained from both the as-synthesized film and an annealed film utilizing Cu K radiation ($\lambda = 1.54059 \text{ \AA}$).

The X-ray diffraction spectrum for the compound (SA₂₅) prepared using X-ray diffraction proved that the (ZnO-4-aminoantipyrine) complex is crystalline in shape because some of the peaks have high intensity because there is more regularity in the direction Proportional to and height of the peak. As for the bonds, they are hydrogen and polarized through hydrogen bonding for the amine group with the

different oxygen atoms on the plate in zinc oxide, as shown in figure (11) as the peaks at 13.56, 15.92, and 27.41 in the Debye-Scherrer equation refer to the following size (25.89, 24.08, and 19.60 nm) respectively, [23] which were calculated from the following equation:

$$D = \frac{K\lambda}{\beta \cos \theta}$$

D = size of minutes, measured in nanometers (nm). K = constant and equal to (0.9). λ = wavelength, equal to (1.5406) Angstrom for the element copper. Converted to the unit of nanometers ($\text{\AA} = 0.1 \text{ nm}$), the wavelength is equal to (0.15406 nm). β = The total width at half the maximum (FWHM) θ = cosine of the diffraction angle and is measured in (deg).

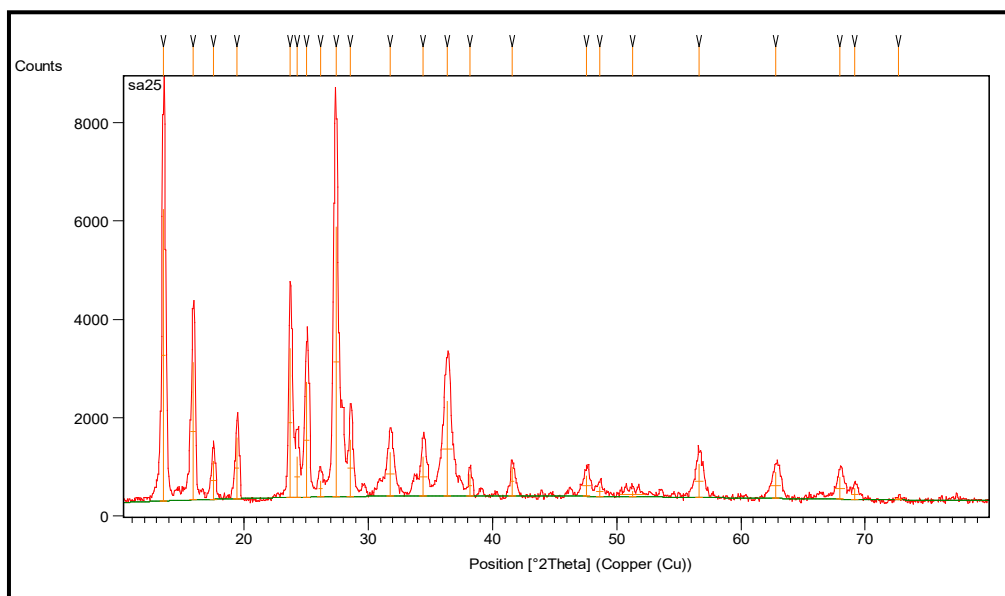


Figure 11: XRD Spectrum for Compound (SA₂₅)

2- Nanocomposites: SA₂₆: In this Nanocomposite, the peaks at 11.79, 13.43, and 34.49 in the Debye-Scherrer equation refer to the following size (53.25, 35.39, and 22.48nm), respectively. 3- Nanocomposites: SA₃₀: The peaks at 13.71, 16.05, and 27.58 indicate

the following Debye-Scherrer equation of (21.05, 25.63, and 19.61nm) respectively. 4- Nanocomposites: SA₃₁: The 11.27, 27.17, and 27.65 peaks indicate the following Debye-Scherrer equation of (24.94, 24.06, and 27.89 nm), respectively.

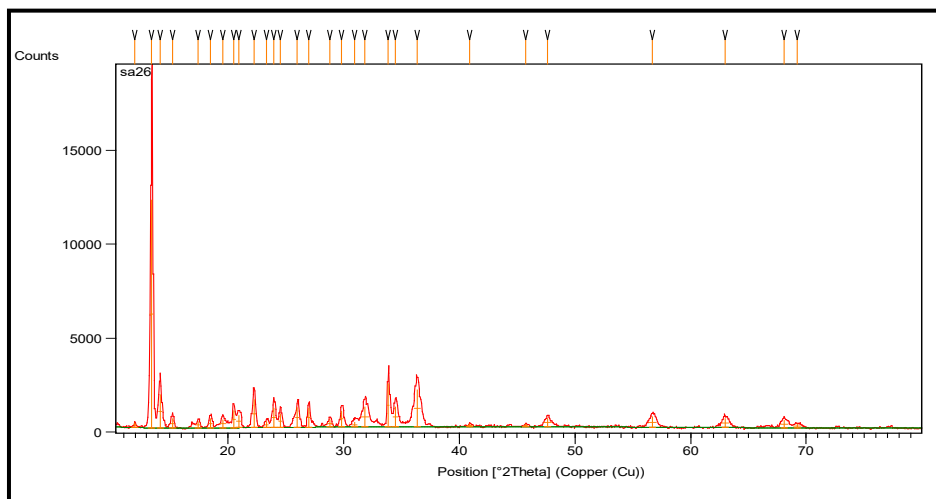


FIGURE 12: XRD Spectrum for Compound (SA₂₆)

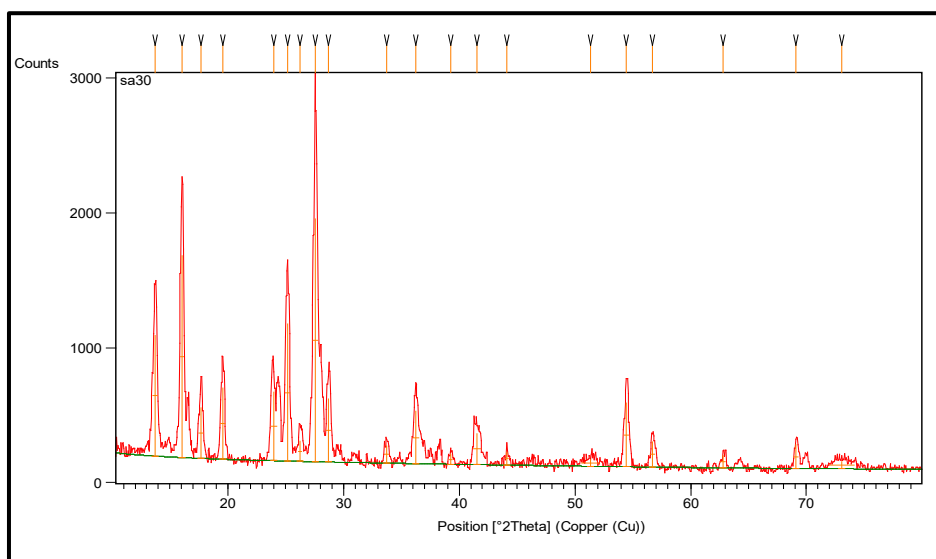


FIGURE 13: XRD Spectrum for Compound (SA₃₀)

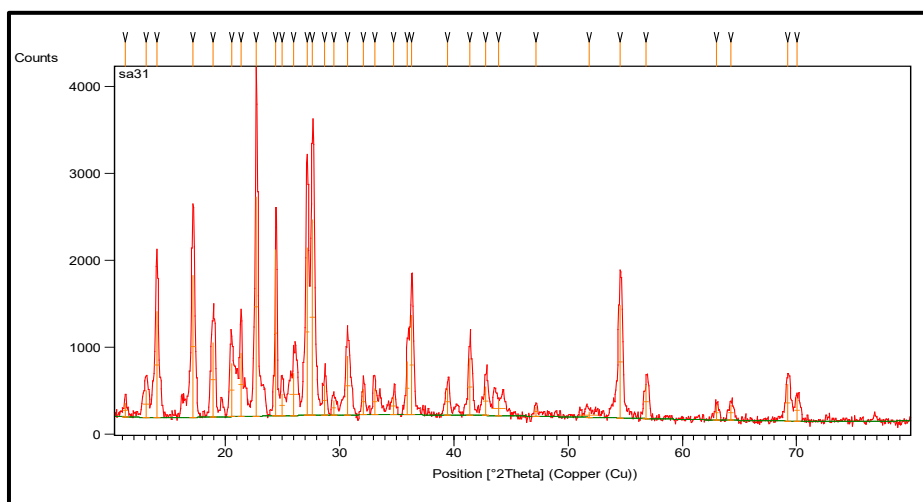


FIGURE 14: XRD Spectrum for Compound (SA₃₁)

Morphology of Nanocomposites

To investigate the surface morphology, particle size, and structure of the composites, FeSEM analyzed the samples; Figures 5, 6, 7, and 8

show the SEM images of these nanoparticles. FeSEM analyzed the samples, and This FeSEM image confirms the nanocomposites' morphology and the current image's apparent sizes [23].

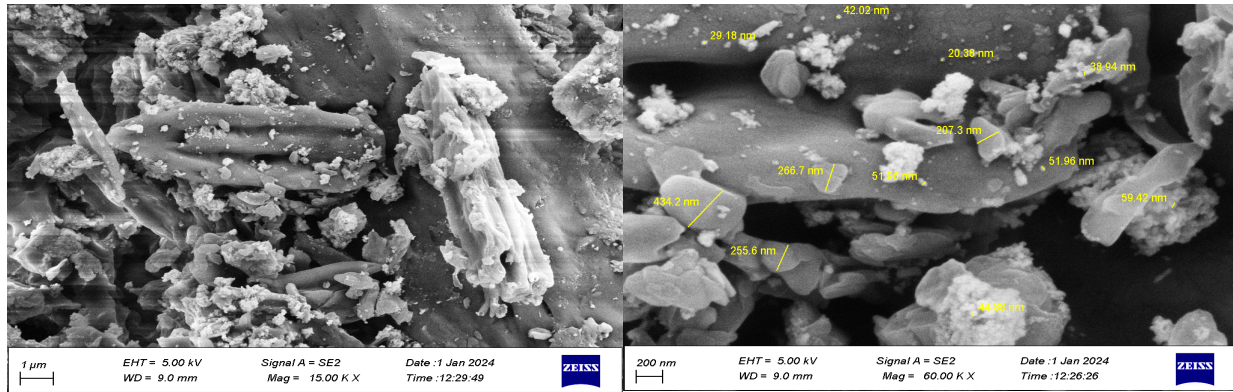


FIGURE 15: FeSEM Image for Compound (SA₂₅)

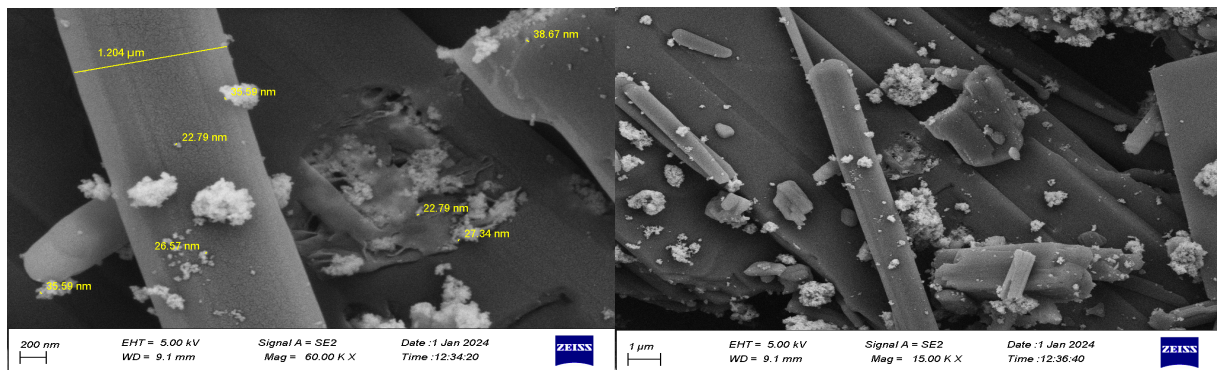


FIGURE 16: FeSEM Image for Compound (SA₂₆)

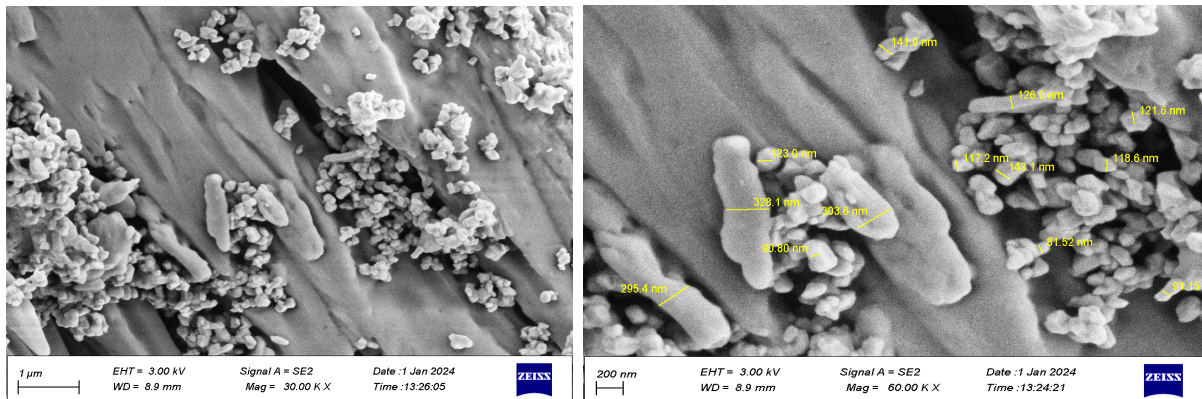


FIGURE 17: FeSEM Image for Compound (SA₃₀)

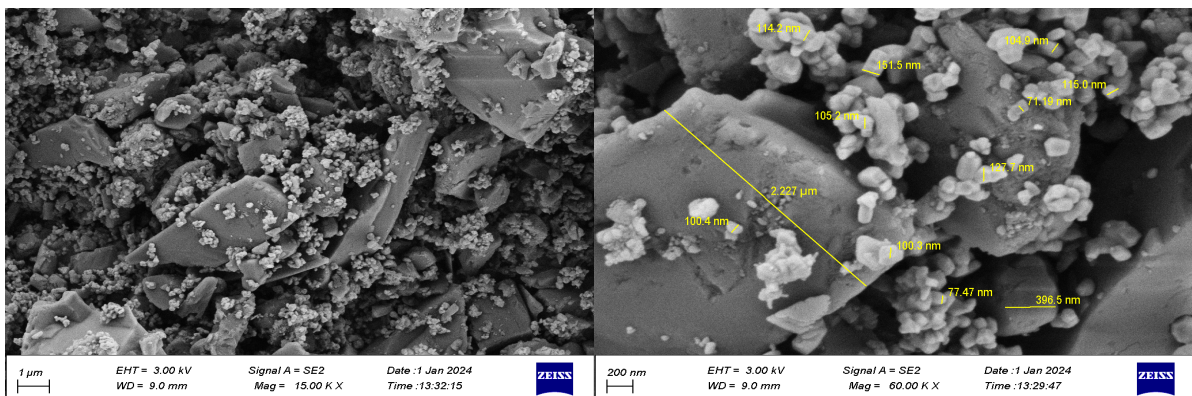


FIGURE 18: FeSEM Image for Compound (SA₃₁)

Enzymatic (Activity and Inhibition)

The following results were obtained from testing the compounds at various concentrations on the alkaline phosphatase enzyme found

in the serum of (someone sick) individual who has an elevated enzyme level at [260.34] IU/L at 37C, compared to the normal range of [32-92] IU/L at 37Co for a healthy individual. As displayed in Table (3).

Table 3: (Activity and Inhibition) of the Enzymes. Units [IU/L]. Concentration [M].

Compounds	1*10 ⁻²	5*10 ⁻³	1*10 ⁻³	5*10 ⁻⁴	1*10 ⁻⁴
4-aminoantipyrine	76.61	75.75	75	74.85	74.7
SA ₃	68.55	74.2	79.55	80.01	81.38
SA ₂₅	67.08	65.19	61.26	48.85	34.82
SA ₂₆	56.45	53.51	51.76	37.25	23.09
SA ₃₀	67.66	63.31	56.36	34.91	1.09
SA ₃₁	52.42	51.29	49.89	44.13	36.22

As the table demonstrates, the produced compound (SA₃) has enzymatic activity. This is because the compounds attach to the allosteric site of enzymes, affecting the enzyme's activity and turning the materials into a catalyst for enzyme activity. Regarding the compound (4-aminoantipyrine, SA₃, SA₂₅, SA₂₆, SA₃₀, SA₃₁), it possesses enzymatic inhibition. This occurs when the produced material attaches to the enzyme's active site, inhibiting the enzyme.

Comprehensive Genus Descriptions

This section has been significantly expanded to include detailed morphological descriptions of each newly identified genus within the Anthomyiidae family. Distinguishing characteristics that differentiate these genera from others are thoroughly detailed, covering aspects such as body size, wing patterns, leg morphology, and antennal structures. To visually support these descriptions, high-quality photographs and illustrations have been incorporated, showcasing key features that aid in genus identification.

Distribution and Ecological Notes

Detailed information on the distribution of the new genera within Iraq is now included, highlighting specific habitat preferences ranging

from semi-arid environments to wetlands. Observations on ecological interactions, such as pollination behaviors, prey-predator relationships, and roles within their respective ecosystems, have been documented. This information is crucial for understanding the ecological significance of these genera and provides a foundation for future biodiversity and conservation efforts.

Biological Activity:

Evaluation of the biological activity of specific synthetic substances. Various generated compounds (4-aminoantipyrine, SA₃, SA₂₆, SA₃₀) were tested for their biological activities using *E. coli*, *Staphylococcus aureus*, and *Candida albicans*. Comparing the outcomes with the conventional antibiotic (4-aminoantipyrine) was done.

By utilizing several quantities of the compounds (5 mg/ml, 10 mg/ml, and 15 mg/ml), they demonstrated that these compounds could inhibit the medium more effectively than the standard antibacterial (4-aminoantipyrine) with a concentration of 10 mg/ml [15]. as displayed in figures (19, 20, 21).

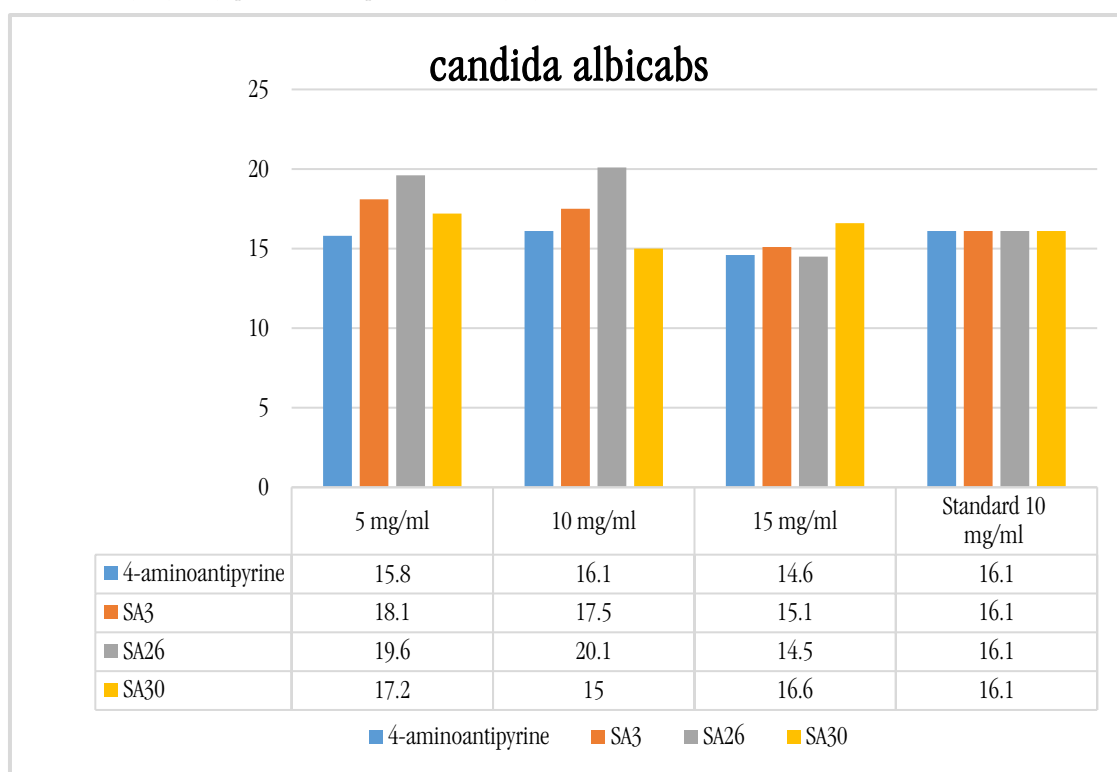
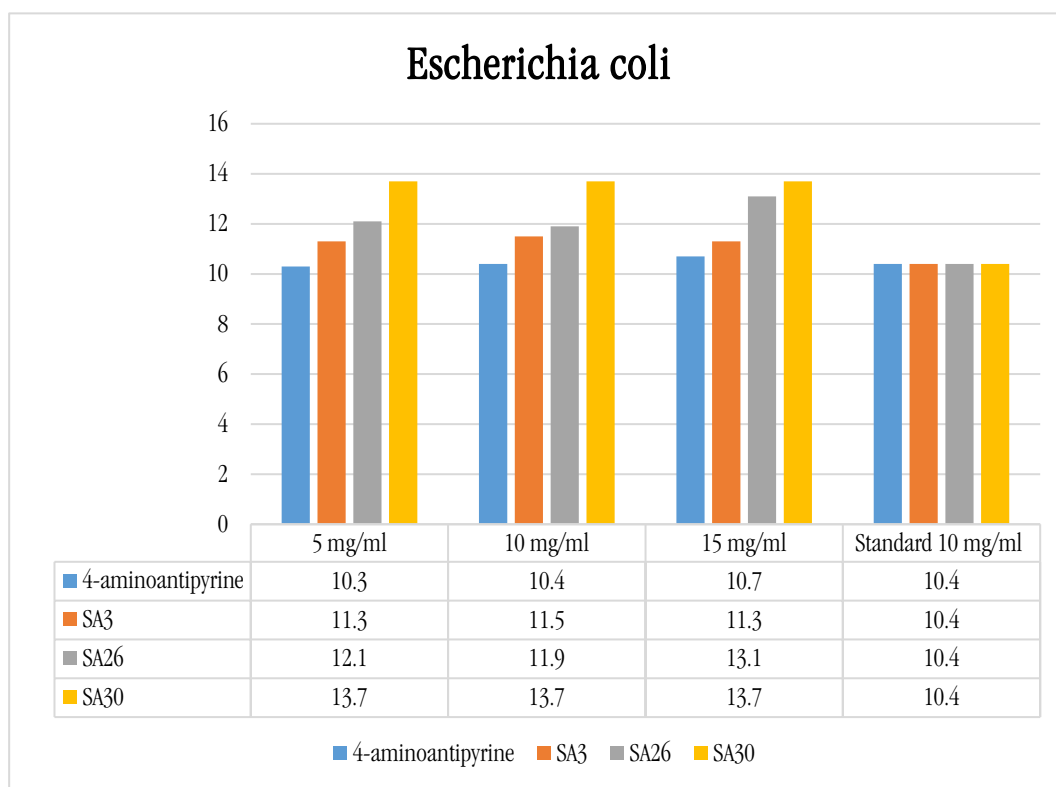
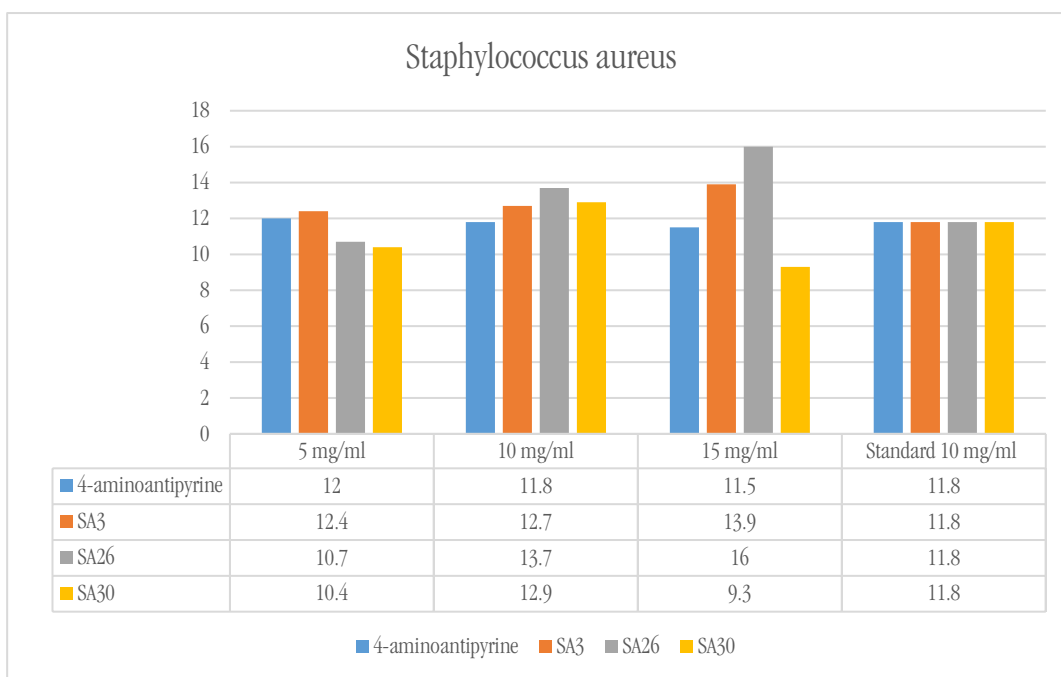


Figure 19: Candida Albicans

**FIGURE 20:** Escherichia Coli**FIGURE 21:** Staphylococcus Aureus

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