

Article

# Synthesis, Characterization, and Biological Activity Evaluation of Graphene Oxide Nano Composites

Hussein Abood Idham\*<sup>1</sup>

1. Directorate General of Education Kirkuk, Kirkuk, Iraq

\*Correspondence: [husain.abood88@gmail.com](mailto:husain.abood88@gmail.com)

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**Abstract:** Graphene oxide (GO) Nano composites can be described as a superior generation of hybrid nanomaterial's with remarkable structural, mechanical, and biological properties. In the current study, the synthesis of graphene oxide nanoparticles was performed through modified Hummers' method from graphite. Afterward, some new functionalized Nano composites (namely H<sub>5</sub>, H<sub>6</sub>, H<sub>7</sub>, H<sub>9</sub>, and H<sub>10</sub>) were fabricated through ultrasonic dispersion and chemical condensation reaction with specific aromatic amine compounds and drug molecules such as mefenamic acid and theophylline. Structural, compositional, and morphological analyses were performed utilizing FT-IR, XRD, FESEM, and TEM techniques. FT-IR spectra indicated that there was a successful functionalization through covalent and non-covalent bonds based on the presence of characteristic vibrations corresponding to hydroxyl, carbonyl, amide, and azomethine groups. The XRD patterns revealed the chemical reduction of GO to rGO sheets due to the disappearance of the reflection peak at  $2\theta \approx 10^\circ$  along with the appearance of well-defined diffractions of (002) planes at  $2\theta \approx 26.15^\circ$ – $27.18^\circ$ . The degree of crystalline ordering was also noted. FESEM and TEM images revealed uniform distribution of well-dispersed drug nanoparticles in the form of ultrafine particles (15.28–39.99 nm) deposited on folded high surface area graphene sheets. Antifungal efficiency was determined by using *Candida albicans* against which antifungal activity was assessed through the agar well diffusion assay. Nano composites H<sub>6</sub>, H<sub>7</sub>, and H<sub>9</sub> displayed prominent antifungal activity at lower concentrations (25 mg/mL) and showed inhibition zones (up to 18.1 mm) which were either better than or equal to the positive control (Gentamycin, 15.3–17.1 mm). It was seen that the increase in concentration resulted in the reduction in efficiency due to steric hindrance and increased viscosity.

**Keywords:** Nanotechnology, Graphene Oxide (GO), Nanocomposites, *Candida Albicans*

## 1. Introduction

Nanotechnology deals with the design, synthesis, and application of materials and systems on the Nano scale ranging from 1 to 100 nanometers (nm) in size, wherein 1 nm is one billionth of a meter. The unique characteristics of nanomaterial's are brought about by the principles of quantum mechanics and high surface-to-volume ratios, different from those of their bulk materials. This leads to innovative applications in the fields of health care, electronics, energy, and environmental sustainability. With its multi-disciplinary approach that combines physics chemistry, biology, materials science, and engineering, nanotechnology has become a key pillar of contemporary innovation. The notion of nanotechnology was initially introduced in a lecture delivered by physicist Richard Feynman in 1959 entitled, the name "Nanotechnology" was first used by Norio Taniguchi in 1974, and further developments in microscopy and materials science have facilitated the

progress of the field. This report presents a comprehensive introduction to nanotechnology, including its basic concepts, history, uses, problems, and future perspectives [1]. Nanotechnology is an area which involves materials and processes on a scale of nanometer. Nano scale means that the measurements of the materials or phenomena involve nanometers. Due to special physical and chemical properties that nanomaterial's possess, the materials with advanced functions can be produced. This opens new horizons in industry where smart materials and processes can be developed [2]. The usage of nanomaterial's is diverse and they are being used in a variety of industries, such as electronics, energy, health care, environmental management, etc. Nano composites that are stronger and more flexible, as well as nanoparticles with improved catalytic activities, are just some examples of how innovation and optimization are taking place because of nanomaterials in industries [3]. There are several forms of graphene-derived materials that have been developed until now such as graphane (hydrogenated graphene), fluorographene (fluorinated graphene), graphdiyne, porous graphene, graphene Nano ribbon (GNR), graphene oxide (GO), and reduced graphene oxide (rGO) [4]. GO and rGO are commonly used for biotechnological purposes, which include theranostics in oncology, imaging agent delivery, and anti-cancer drug carriers [5]. Graphene oxide (GO) is usually formed using modified Hummer's process, which is the oxidized form of graphene. Reduced graphene oxide (rGO) is formed by adding appropriate reducing agents to GO [6]. Overall, graphite, diamond, and amorphous carbon are categorized based on the structural arrangement of carbon atoms. Graphene consists of 2D carbon atoms having an ultra-thin layer of atom sheets. The nanomaterial's are directly related to the material as graphene is one of the basic components of the nanomaterial's [7]. Graphene has also emerged as an important material in several industrial applications, such as energy conversion, energy storage along with photocatalysis [8]. Graphene is available in various forms and are primarily used for heterogeneity generation in several applications. They can also be useful in developing the redox vibrational medium as well as water purification catalysts due to their large surface area and catalytic, optical and electrical properties [9]. Extremely porous and larger surface areas of the carbon nanoparticles also have high adsorption properties that can offer active sites for wastewater treatments [10]. The unique characteristics of graphene are its high carrier mobility that is approximately  $200,000 \text{ cm}^2/\text{V/s}$  [11], mechanical flexibility with its Young's modulus being about 1100 GPa and the fracture strength 125 GPa [12], high chemical/thermal stability [13], transparency [14], large surface area with its value estimated at  $2630 \text{ m}^2/\text{g}$  [15] and good electrical and thermal conductivity, approximately  $5000 \text{ W/m/K}$  [16]. The chemical reduction approach that includes sonicating flakes of graphite (Gt) to form GO and then reducing it provides for the inexpensive production of bulk graphene nanosheets. However, the application of extremely dangerous  $\text{NaBH}_4$  or hydrazine as reductants of GO requires one to be especially cautious [17]. The use of biological applications of graphene-based materials has been extensively researched owing to their biocompatibility, unusual conjugation properties, and relative cheapness [18]. The biological uses of graphene including DNA hybridization device, cancer treatment [19]. It also possesses a vast potential application in many other fields of biology like antibacterial properties, therapeutic properties, and biosensing. Recent studies have shown the antimicrobial property of rGO [20]. conducted experiments to demonstrate the antibacterial property against E.coli bacteria using Gt, graphite oxide (GtO), GO, and reduced GO (rGO). It was found that their antibacterial activity was much higher than that of GtO, and GO exhibited maximum antibacterial activity. It was also observed that the antibacterial activity was concentration dependent [21].

## 2. Materials and Methods

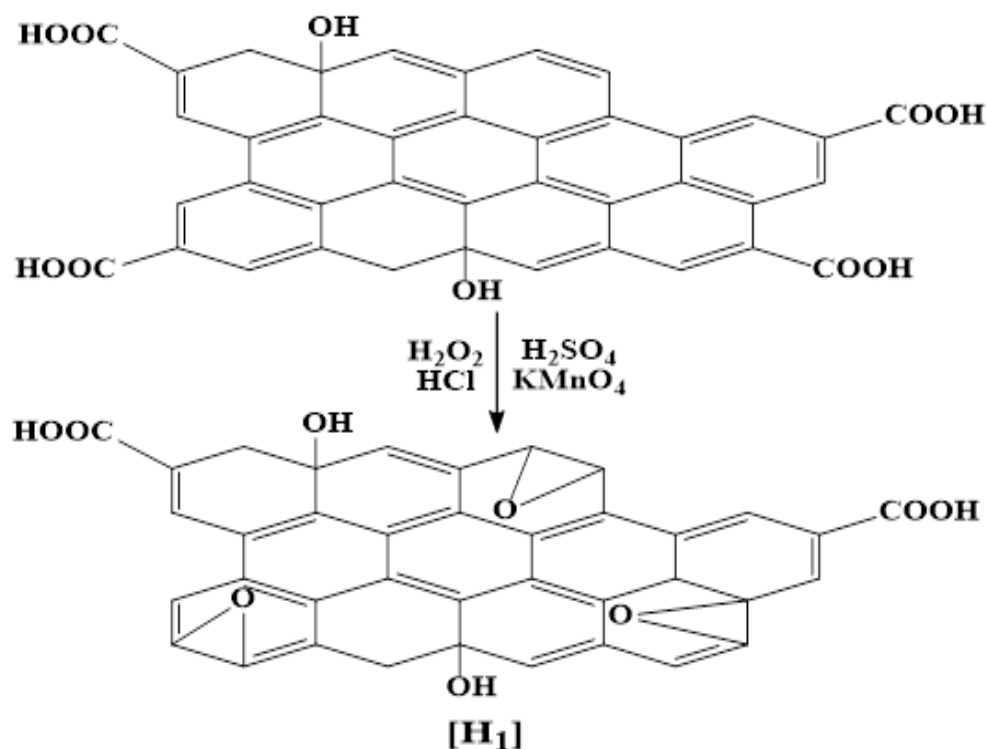
### 2.1. Materials

All chemicals in their pure form were obtained from Fluka and Merck Company. Characterization was done based on comparing the physical properties of the substances with samples in literature. The Fourier Transform Infra-Red Spectroscopy was done using Shimadzu FT-IR 8400S spectrometer in the range  $400\text{--}4000\text{ cm}^{-1}$  by the use of pressed KBr discs, Centre fuge Hettich, 6000 U/min, Germany (Ultra-sonic water bath). Information regarding the surface structure, inter particle distances and particle sizes were obtained through FESEM which is field emission scanning electron microscopy. This was carried out using TESCAN (Belsorp Mini II / Czech Republic) at the University of Kashan, Iran. "A Shimadzu XRD-6000 X-ray diffract meter was used for material analysis with a nickel filtered copper X-ray source".

### 2.2. Methods

#### 2.2.1. Graphene Oxide Nanoparticles Synthesis<sup>(22)</sup> [H<sub>1</sub>].

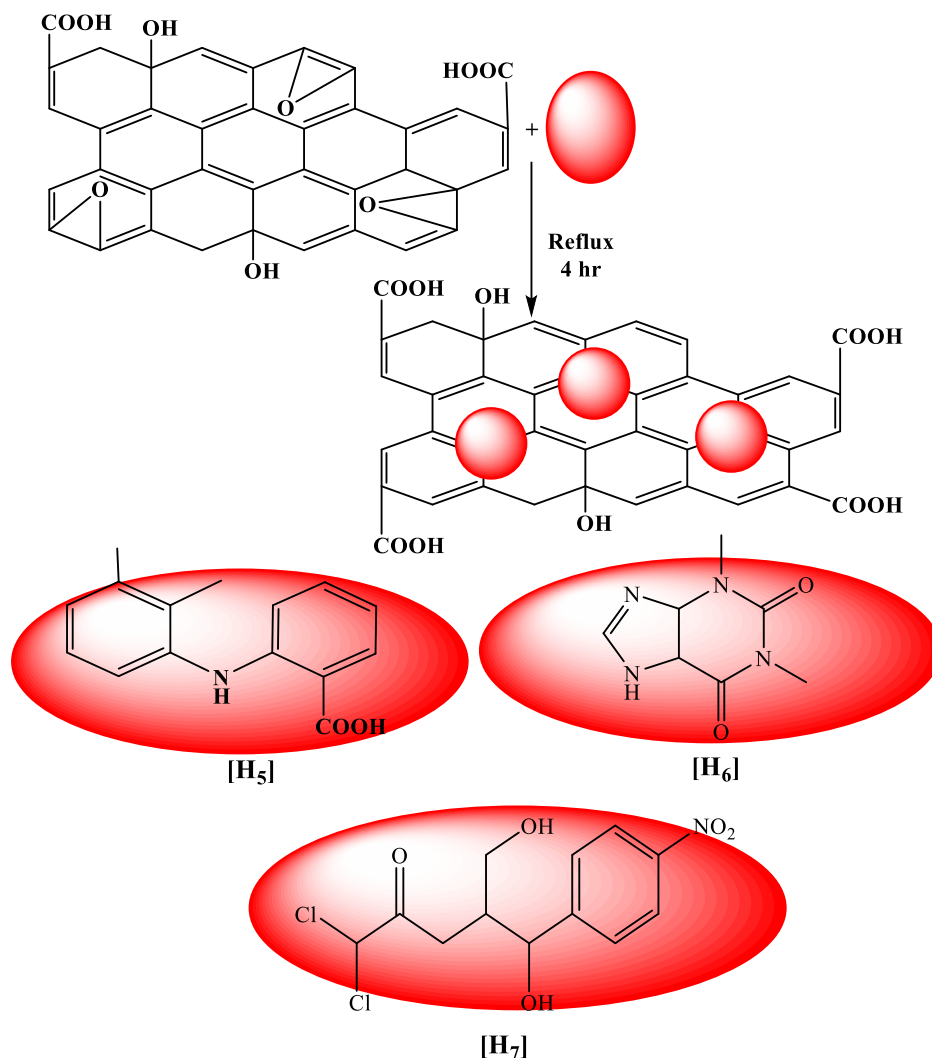
A 600 mL flask was put into the ice bath and 46 mL of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) was added to it with stirring using a magnet at  $0\text{ }^\circ\text{C}$ . After that, 0.017 mol (1.45 g) of sodium nitrate was added to the flask slowly over 15 minutes with stirring. After that, 1 g of graphite was added to the flask slowly over 10 minutes. Then, 0.03 mol (4.74 g) of potassium permanganate was added slowly and carefully to the flask over 10 minutes keeping the temperature lower than  $10\text{ }^\circ\text{C}$  for two hours. The solution was then taken out of the ice bath and 46 mL of distilled water was added dropwise while stirring for 15 min at  $98\text{ }^\circ\text{C}$ . Warm distilled water, with a temperature less than  $50\text{ }^\circ\text{C}$ , was added and stirring continued for 60 minutes. Finally, 12 mL of 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added while stirring for 30 min and After this, the solution was split into two portions, 150 mL of distilled water was added to each portion, and the solution was allowed to precipitate for 24 hours. The supernatant was decanted off, and the precipitate was washed with 30 mL of 10% HCl once, and with deionized water five times (30 mL each) until the pH became neutral. Then, the solution was dried at  $60\text{--}70\text{ }^\circ\text{C}$ .



Scheme 1: Synthetic of Graphene Oxide Nanoparticles

### 2.2.2. Preparation of Graphene Oxide Nanocomposites [H<sub>5</sub> - H<sub>7</sub>]

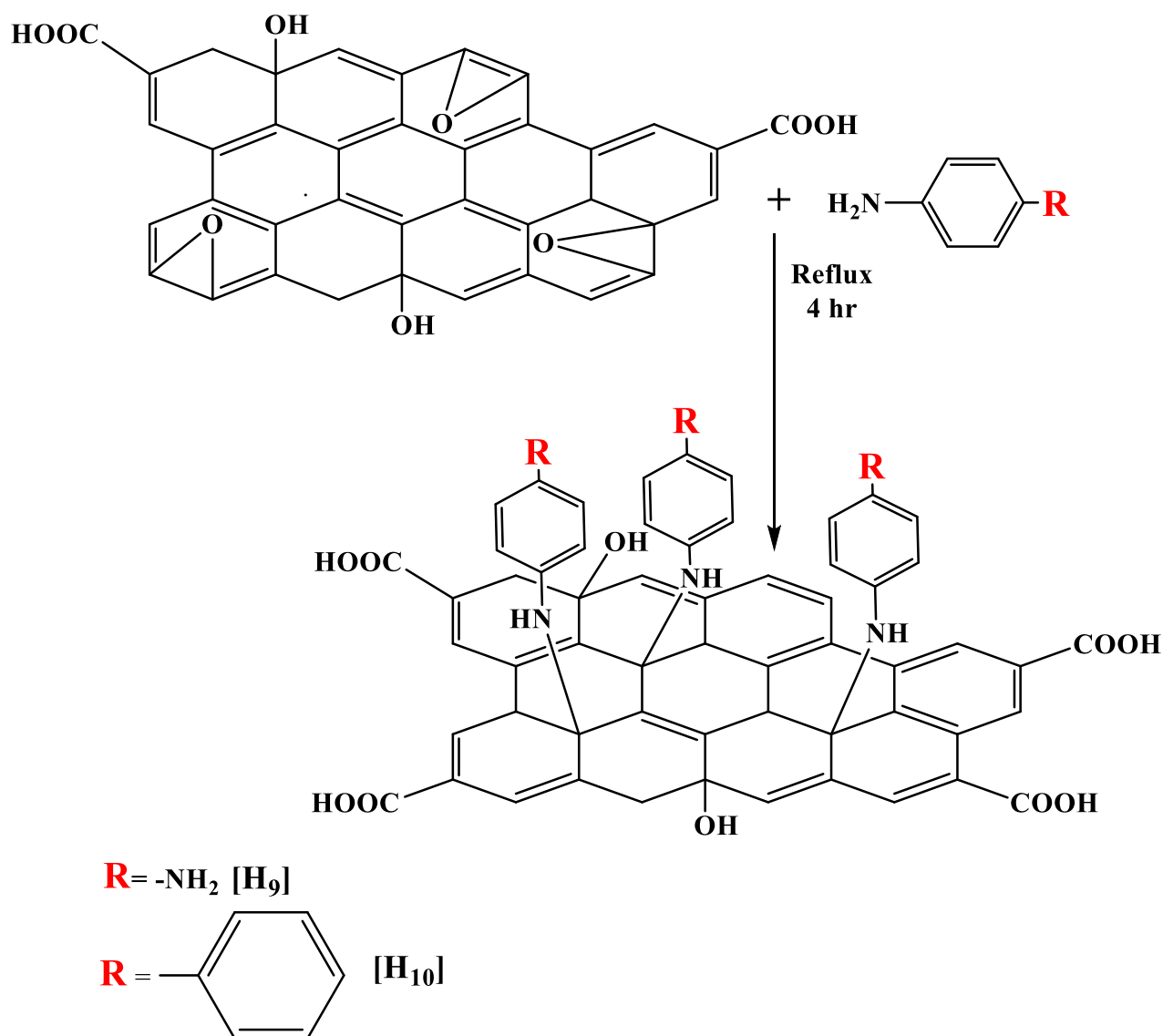
0.4 mol of the prepared graphene oxide nanoparticles [H<sub>1</sub>] was introduced into 10 mL of distilled water, where ultrasonication at 50 Hz frequency was applied for 30 minutes. After that, 0.4 mol of a chosen aromatic amine was added to the mixture, and refluxing at 100 °C temperature lasted for 4 hours. The product was isolated, purified by washing with 10 mL deionized water three times and dried at 80 °C until constant weight.



**Scheme 2:** Synthetic of Graphene Oxide Nanoparticles[H<sub>5</sub>-H<sub>7</sub>]

### 2.2.3. Preparation of Graphene Oxide Nanocomposites [H<sub>9</sub> - H<sub>10</sub>]

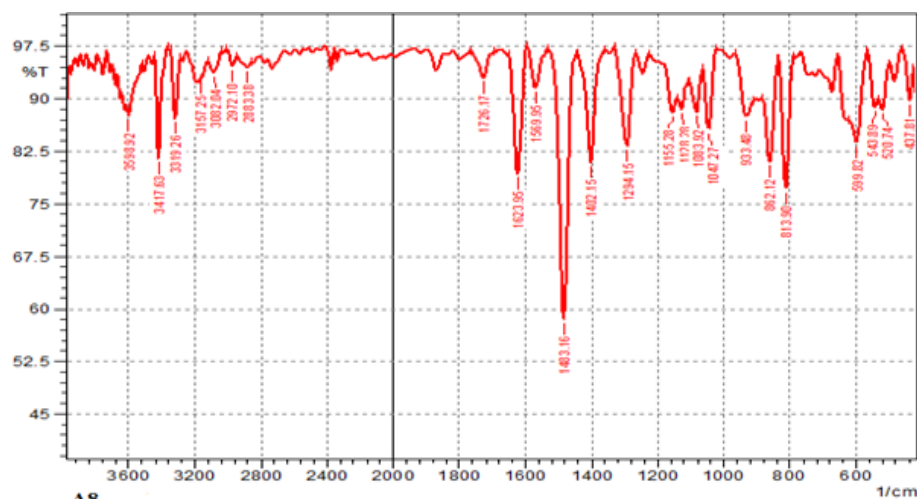
0.2 mole of synthesized graphene oxide nanoparticles [H<sub>1</sub>] was dissolved in 10 mL distilled water and ultrasonically for 30 minutes at a frequency of 50 Hz. Subsequently, 0.2 mole of chosen aromatic amine was introduced and kept heated for 4 hours at 100 °C. It was then filtered out and washed thrice with 10 mL of deionized water, followed by drying at 80 °C in the oven until a constant weight was obtained.



**Scheme 3:** Synthetic of Graphene Oxide Nanoparticles[H<sub>9</sub>-H<sub>10</sub>]

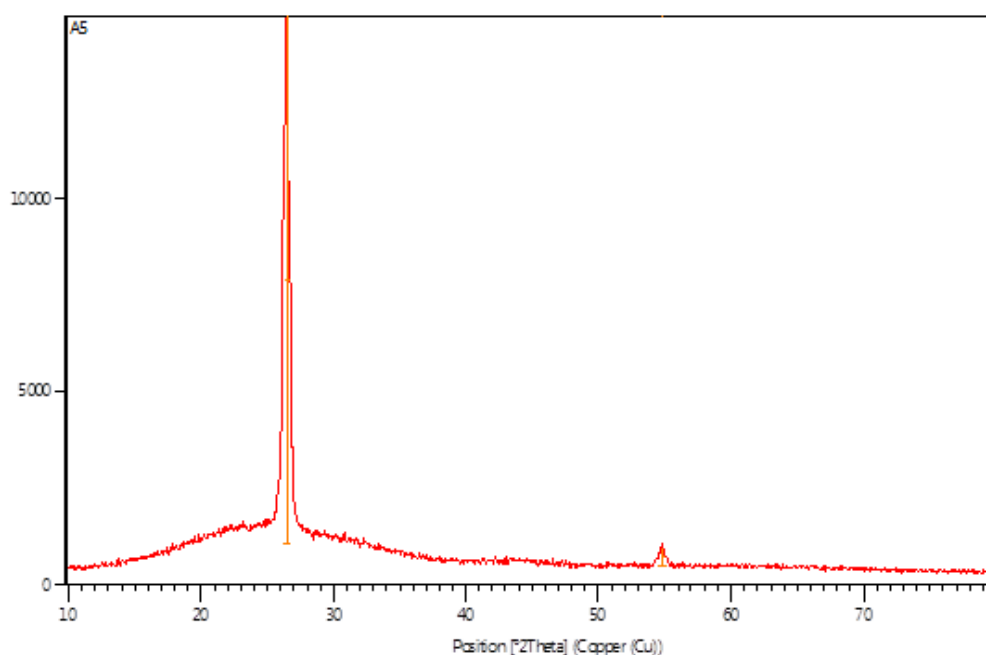
### 3. Results and Discussion

In this paper The preparation of graphite oxide (GO) from graphite made the latter a precursor for synthesis of other Nano composites. Later on, Nano composites [H<sub>5</sub>-H<sub>7</sub>] were obtained through the addition of graphene oxide (GO) nanoparticles to one of the drugs represented in Scheme 2. Reduction takes place through the transformation of carbon hybridization from  $\text{sp}^3$  to  $\text{sp}^2$ . Reduced graphene oxide Nano composites are described as different from the original graphene oxide nanoparticles; as a result, the properties of the latter, including electrical, thermal, mechanical and surface morphology are not the same as those of the former. FT-IR spectrum for compound H<sub>5</sub> revealed the existence of a unique peak at  $3598 \text{ cm}^{-1}$ , which refers to the combined hydroxyl groups (O-H) of the alcohol and carboxylic acid functional groups. Another peak found at  $3319 \text{ cm}^{-1}$  belongs to the stretching vibrations of the N-H group. Also, two other peaks were identified at  $1726 \text{ cm}^{-1}$   $1294 \text{ cm}^{-1}$ ; they belong to the vibrations of the carbonyl (C=O) and (C-O) groups of carboxylic, respectively. Furthermore, another two peaks were recognized at  $1623 \text{ cm}^{-1}$   $1483 \text{ cm}^{-1}$ , belonging to the vibrations of the double bond (C=C). In addition, one peak exists for the aromatic C-H group at  $3082 \text{ cm}^{-1}$ , and there are peaks for the stretching and bending vibrations of the aliphatic C-H group at  $2972 \text{ cm}^{-1}$   $1155 \text{ cm}^{-1}$ , respectively.



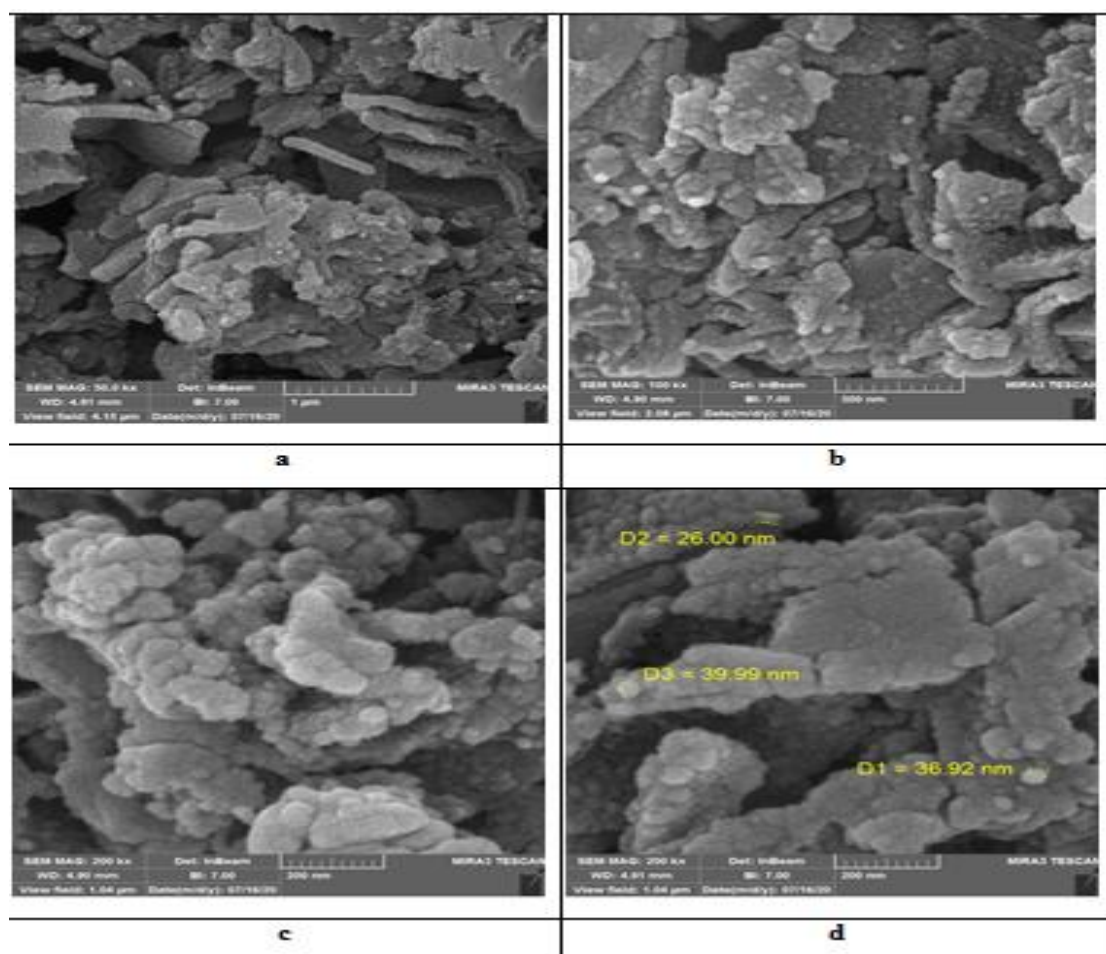
**Figure 1.** Shows the FT-IR spectrum for compound **H<sub>5</sub>**.

The X-ray diffraction (XRD) analysis of the **H<sub>5</sub>** composite displays structural properties of the Nano composite that consists of graphene oxide decorated with mefenamic acid (GO + Mefenamic acid). The spectrum shows a highly sharp and intense diffraction peak centered at the  $2\theta$  value of approximately  $26.4^\circ$ , which is related to the specific (002) crystalline planes of graphite structure or reduced graphene oxide (rGO). The clear appearance of such diffraction peak along with the absence of usual graphene oxide diffraction peak that can be detected at a lower  $2\theta$  value of approximately  $10^\circ$  suggests partial reduction or restacking of graphene oxide due to its interaction and binding with mefenamic acid molecules. As a result, the decrease in interlayer spacing  $d$  occurred because of the removal of large oxygen-containing functional groups or the effect of strong  $\pi$ - $\pi$  interaction between aromatic ring of the mefenamic acid and the carbon lattice. Moreover, there is a small weak intensity peak that appears at a  $2\theta$  value of approximately  $54.5^\circ$  corresponding to the diffraction plane (004), thus validating the existence of long-range periodic structure along the crystallographic  $c$ -axis. The sharpness and intensity of the main peak exceeding 13,000 counts indicate that the degree of crystallization and ordering of the structure of the resulting hybrid are restored to a high level. This allows concluding that mefenamic acid was successfully loaded on graphene oxide.



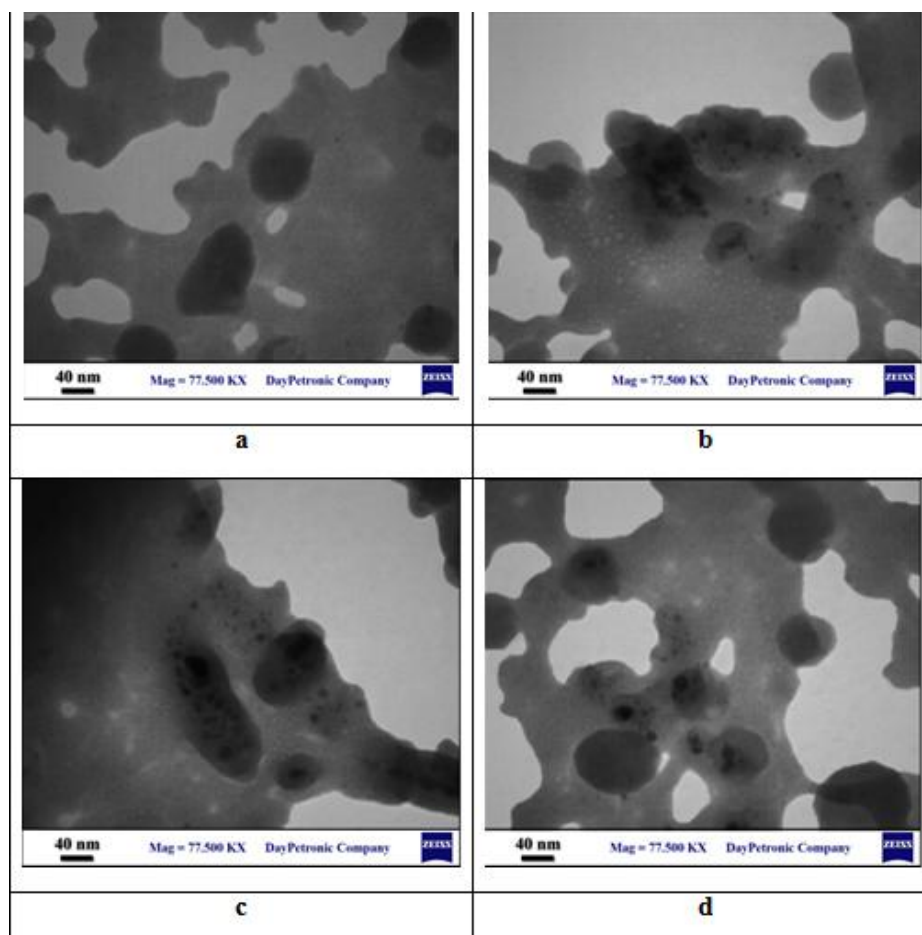
**Figure 2.** Shows the XRD spectrum for compound **H<sub>5</sub>**.

Field Emission Scanning Electron Microscopy (FESEM) micrographs of the H5 composite, which is the hybrid structure of the graphene oxide loaded with mefenamic acid (GO + Mefenamic acid), can be seen from the Field Emission Scanning Electron Microscopy (FESEM) micrographs shown below. Upon careful observation of the FESEM micrographs at lower and intermediate magnifications (a and b), it can be observed that there is a distinct stacking of layers indicative of the presence of graphene oxide (GO) sheets. The GO sheets have random orientation and cluster formation owing to interfacial interaction and van der Waals forces. With regard to the higher magnifications (c and d), there is the clear presence of densely-packed ultrafine grains and nanoparticles in the GO sheets.



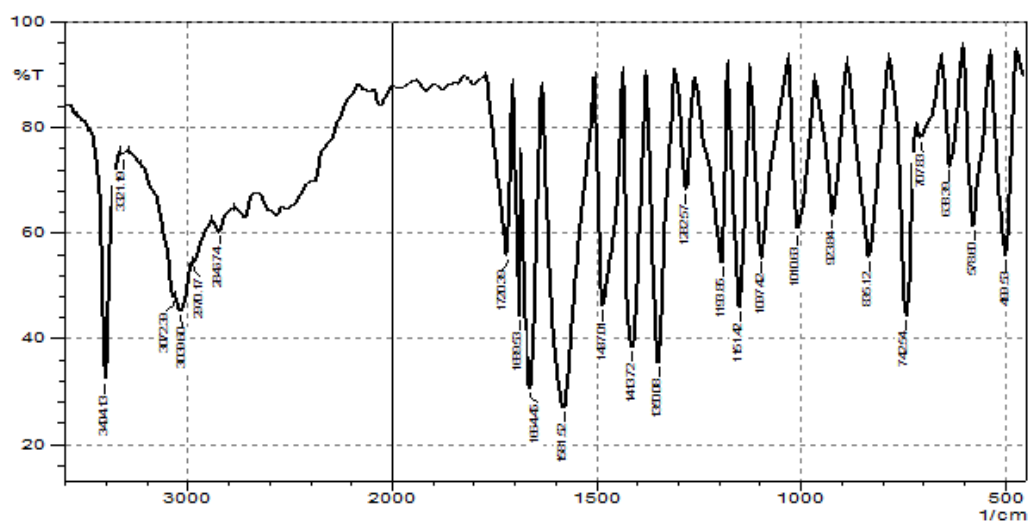
**Figure 3.** Shows the FESEM for compound H<sub>5</sub>

The transmission electron microscopy (TEM) images shown in the figure below depict the structure and morphology of the mefenamic acid-loaded graphene oxide composite (GO + Mefenamic acid). Visual examination of the image (a) indicates the typical sheet-like and lamellar nature of the graphene oxide (GO), which is observed as thin, semi-transparent sheets with wavy edges and naturally occurring interstitial voids created during the process of fabrication. As we move on to image (b, c, and d), there is a clear change in optical density, along with the presence of dark spots and particles. These particles are uniformly dispersed and firmly attached throughout the surface and within the folds of the graphene sheets. These dark spots and particles correspond to the loading of mefenamic acid molecules onto the graphene oxide sheets, wherein the dark spots are a consequence of high electron density of the assembled molecules relative to the thin graphene sheets.



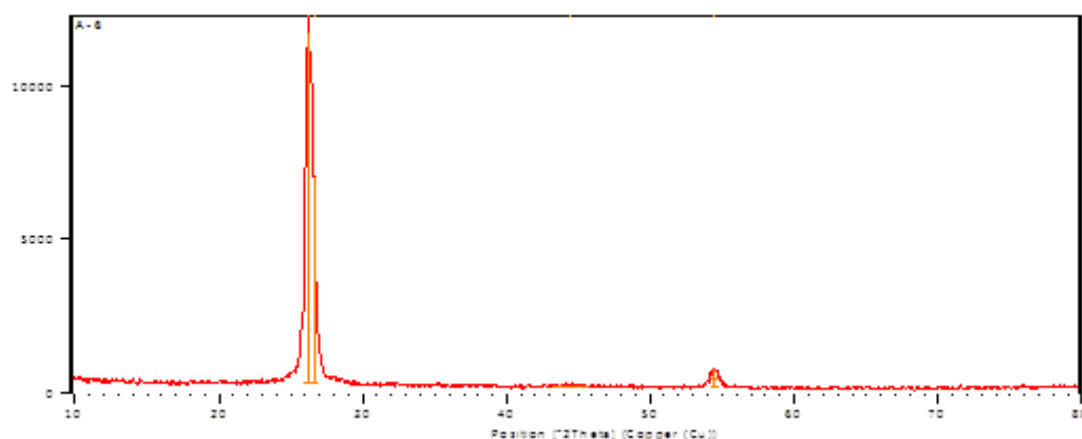
**Figure 4.** Shows the TEM for compound **H<sub>5</sub>**

Compound **H<sub>6</sub>** showed an FT-IR spectrum with absorption peaks at  $3404\text{ cm}^{-1}$ , which is attributed to the overlapped hydroxyl (O-H) groups of the alcoholic and carboxylic functions. Absorption peaks were also observed at  $1720\text{ cm}^{-1}$  and  $1282\text{ cm}^{-1}$ , due to the stretching of the carboxylic carbonyl (C=O) and (C-O) bonds, respectively. In addition, there is an absorption peak at  $1689\text{ cm}^{-1}$ , due to the stretching of the amide carbonyl (C=O) bond, whereas an absorption peak at  $1664\text{ cm}^{-1}$  is attributed to the stretching of the azomethine (C=N) bond. Two more absorption peaks were observed at  $1581\text{ cm}^{-1}$  and  $1487\text{ cm}^{-1}$ , due to the stretching of the (C=C) bonds. There is also an absorption peak at  $3072\text{ cm}^{-1}$  for the aromatic (C-H) stretching vibration.



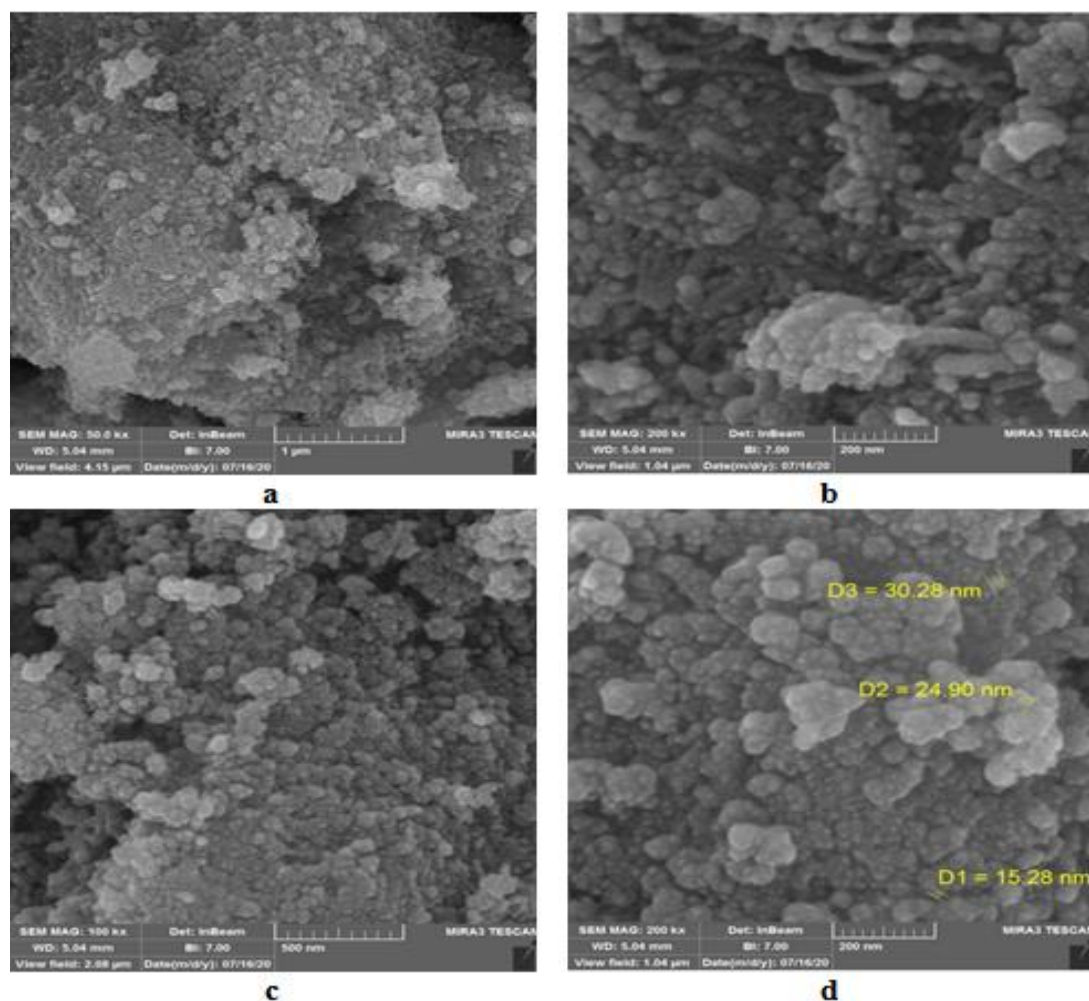
**Figure 5.** Shows the FT-IR spectrum for compound **H<sub>6</sub>**.

The X-ray diffraction (XRD) pattern presented in the first figure contains a strong diffraction peak at the 2theta angle equal to about 26.5° and a weak additional peak at 54.5°. These two peaks relate to the crystalline planes (002) and (004) of the hexagonal carbon lattice formed by restacking the graphene layers. It is important to notice the absence of any peak specific for graphene oxide (GO) with the angle of about 10° due to increased distance between the layers caused by the oxygen-containing functional groups. The absence of this peak is indicative of the successful rearrangement and restacking of the graphene layers due to the influence of theophylline molecules shown in the second figure. Consequently, the interlayer distance decreased to approximately 0.34 nm under the action of attraction forces and stable binding sites. In addition, the absence of the sharp crystalline peaks typical for pure theophylline in this pattern clearly confirms scientifically that the drug does not have a crystalline form. Instead, it is highly molecularly dispersed or amorphous, Successfully incorporated into the surface and interlaminar regions of the graphene oxide nanosheets through interlayer pi-pi interactions and hydrogen bonding with the nearby carboxyl and hydroxyl groups, thus proving the formation of the uniform composite structure.



**Figure 6.** Shows the XRD spectrum for compound H<sub>6</sub>.

In addition, field-emission scanning electron microscopy (FESEM) micrographs of graphene oxide-based hybrid Nano composites (GO + Theophylline) show that the morphological structure is uniform and has a rough surface topography whereby the material exists as granular or quasi-spherical nanoparticles which are densely packed in the surfaces and folds of the graphene oxide flakes. In the FESEM micrographs (a, b, and c), it can be observed that the polarized functional groups in the graphene oxide framework (for example carboxyl and hydroxyl groups as shown in the structural model) were suitable for acting as an effective supporting medium and effective binding sites, which enhanced stability of theophylline molecules and minimized agglomeration of the molecules into bigger sizes. This is supported by the high-resolution FESEM image (d), where measurements of the diameters of the particles confirm the Nano scale dimensioning with distinct Nano scale size of approximately between 15.28nm to 30.28nm (D1= 15.28nm; D2= 24.90 and D3= 30.28nm).



**Figure 7.** Shows the FESEM for compound **H<sub>6</sub>**

The transmission electron microscopy (TEM) provided herein are indicative of the accurate structural and morphological identification of the functionalized graphene oxide composite doped with theophylline molecules (GO + Theophylline). The image obtained is an evidence of the structural hybridization of the materials through which a layered structure was formed. This structure is composed of thin and flexible graphene oxide sheets that have surface wrinkles and folds providing high specific surface area for the composite. In terms of optical density, there are clear indications of dark regions and uniformly distributed nanoparticles on the surface and within the inter-laminar spaces, thereby proving the efficient incorporation of theophylline molecules within the structure. This efficient morphological integration is due to the strong physico-chemical interactions between the two components, including  $\pi - \pi$  interactions between aromatic rings of theophylline and the hexagonal structure of graphene oxide. There are also effective hydrogen bonds between the theophylline molecules and the polar functional groups, such as hydroxyl and carboxyl groups present along the surface and edges of graphene oxide.

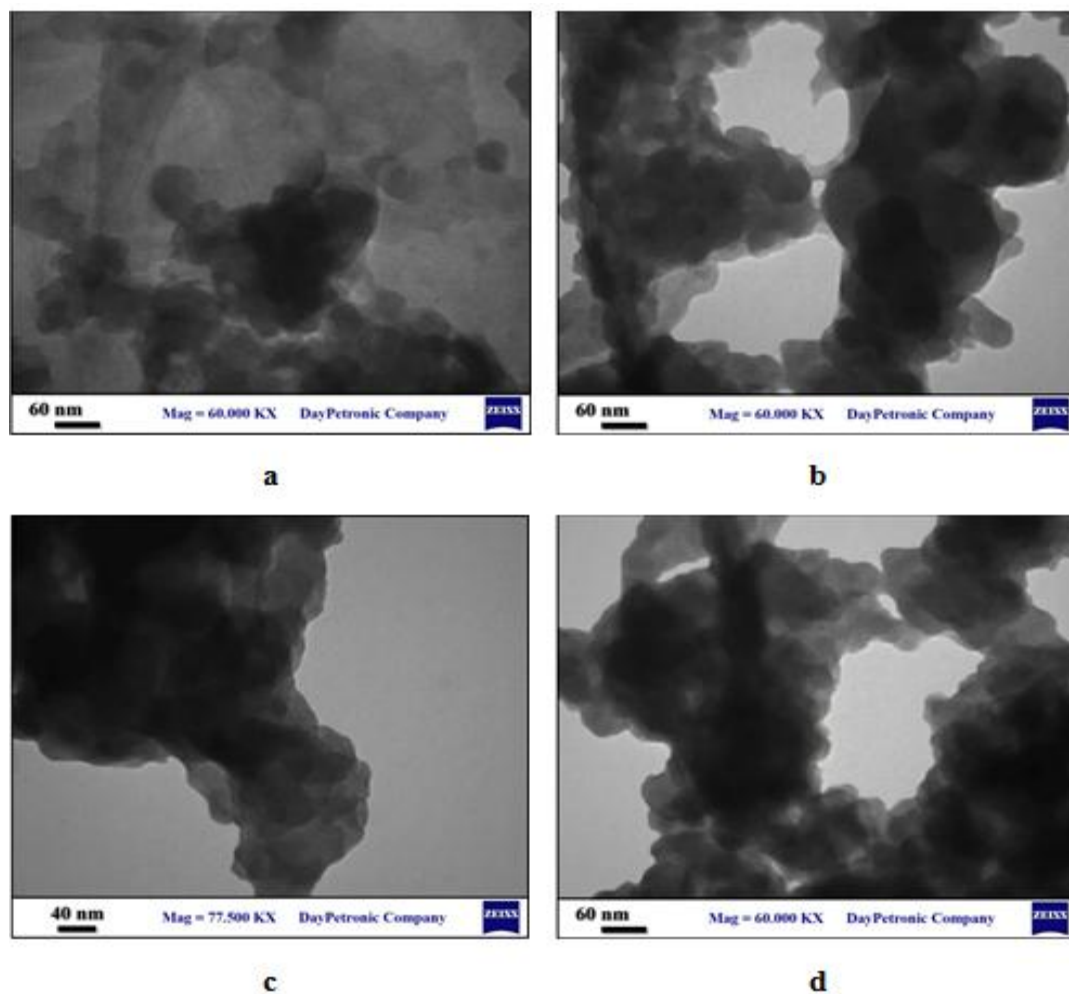


Figure 8. Shows the TEM for compound  $H_6$

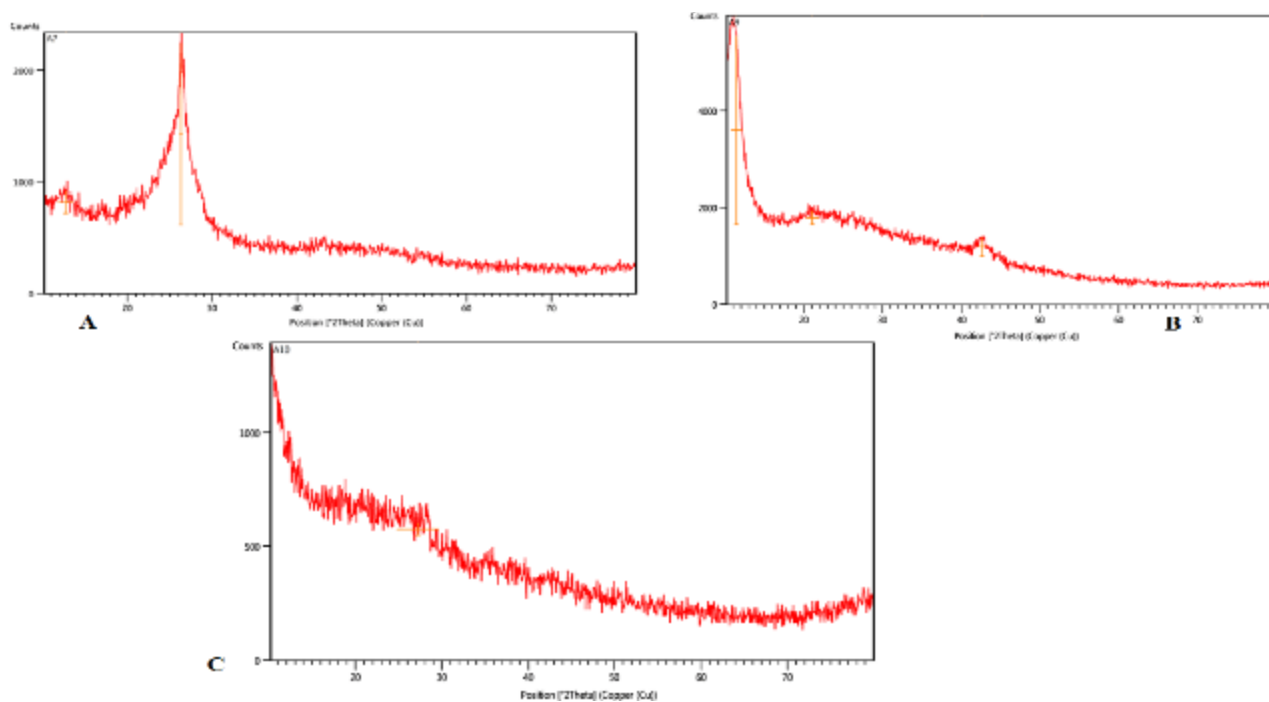
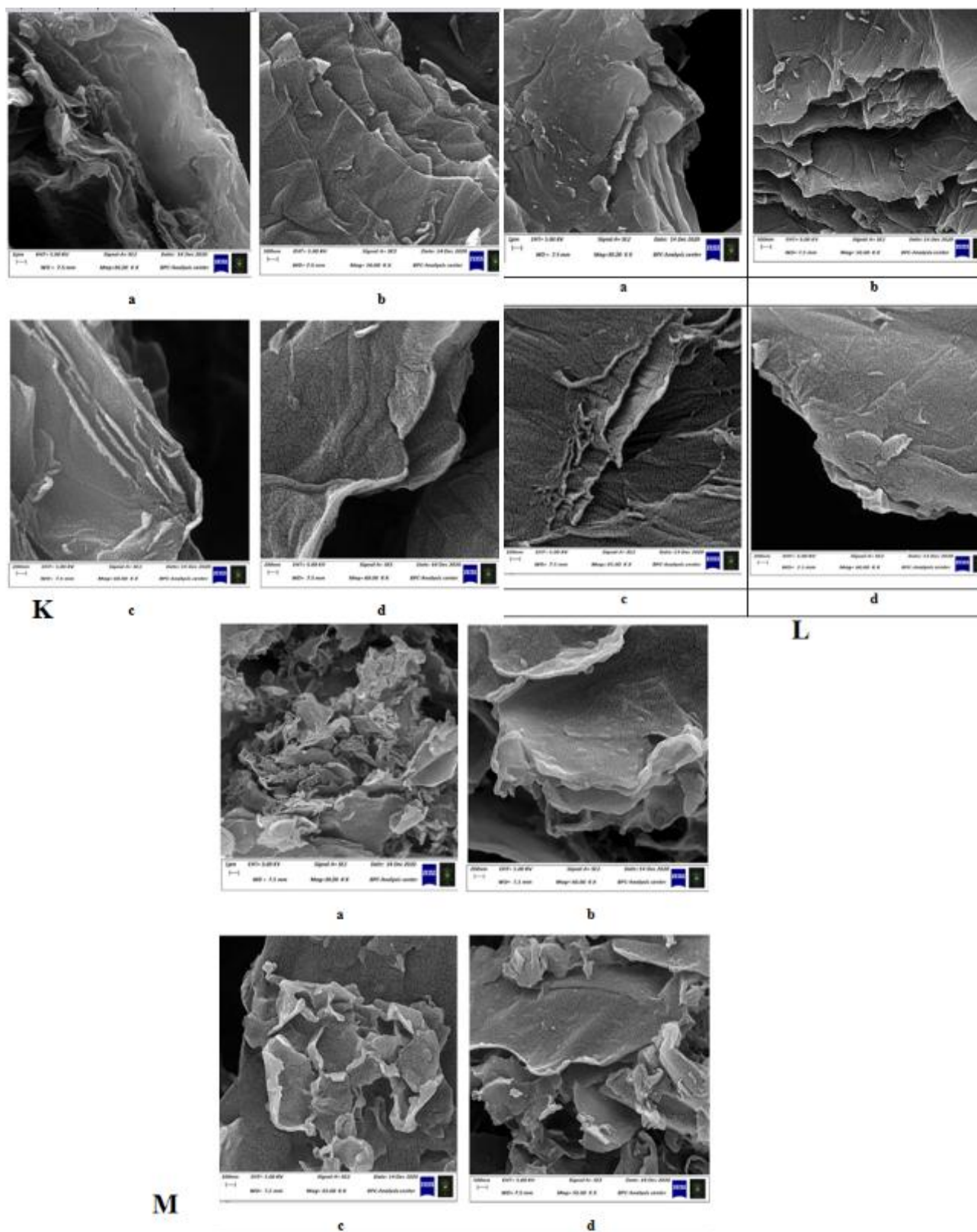


Figure 10. Shows the XRD spectrum for compound [ $H_7$ (A),  $H_9$ (B) &  $H_{10}$ (C)].



**Figure 11.** Shows the FESEM for compound [H<sub>7</sub>(K),H<sub>9</sub>(L) &H<sub>10</sub>(M)].

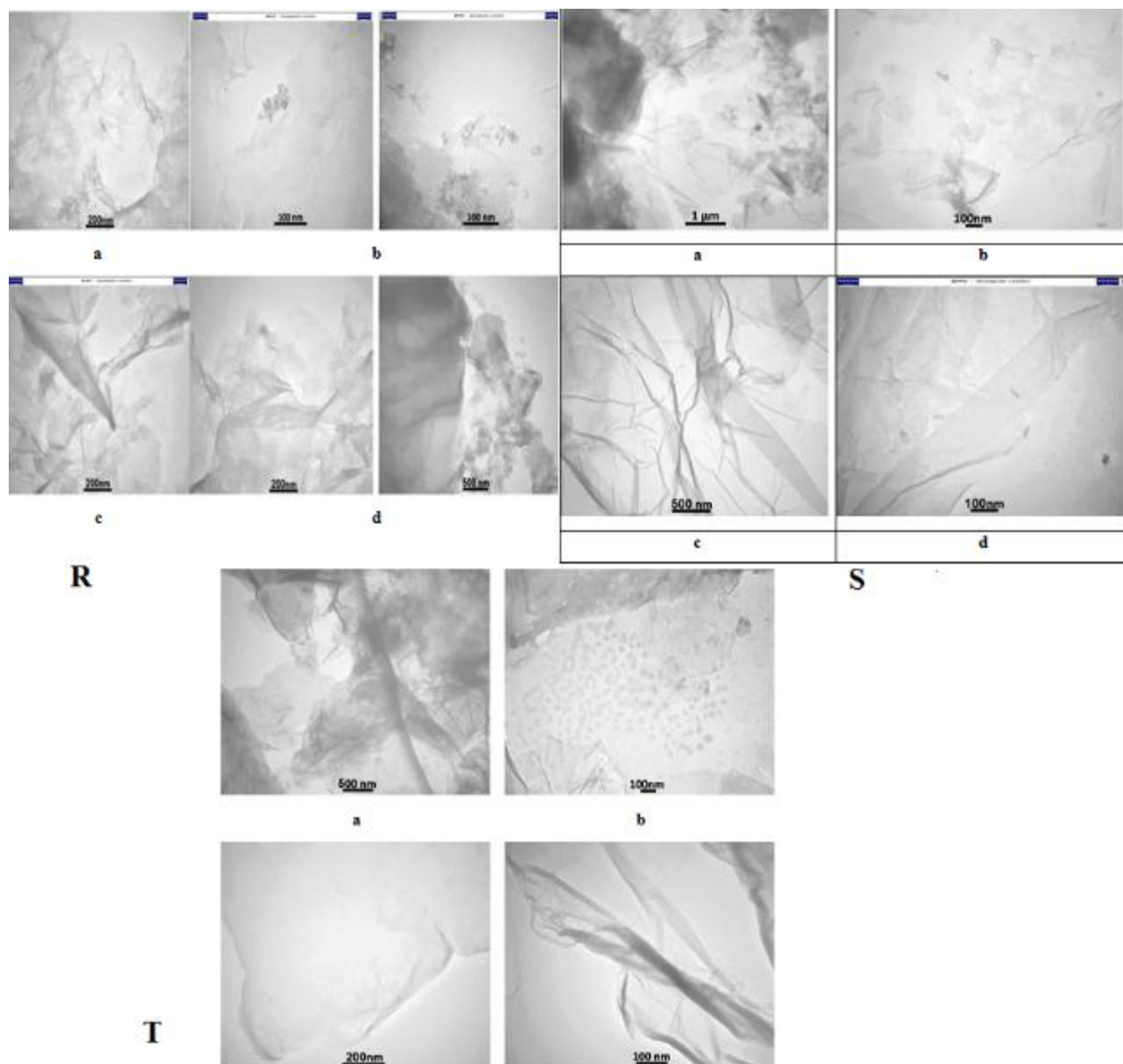


Figure 12. Shows the TEM for compound [H<sub>7</sub>(R),H<sub>9</sub>(S) &H<sub>10</sub>(T)].

Table 1. FT-IR characteristic absorption for compounds [H<sub>7</sub>,H<sub>9</sub> and H<sub>10</sub>]

| Comp            | $\nu\text{NH}$ (cm <sup>-1</sup> ) | $\nu\text{CH}_{\text{alph}}$ (cm <sup>-1</sup> ) | $\nu\text{C-H}_{\text{Arom}}$ (cm <sup>-1</sup> ) | $\nu\text{OH}$ (cm <sup>-1</sup> ) | Others (cm <sup>-1</sup> )                                                                                                |
|-----------------|------------------------------------|--------------------------------------------------|---------------------------------------------------|------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| H <sub>7</sub>  | 3321                               | 2921                                             | 3082                                              | 3417                               | C=C <sub>Ar</sub> (1568-1438)<br>C=O(1622)                                                                                |
| H <sub>9</sub>  | 3303                               | 2904                                             | 3109                                              | 3375                               | C=O(1600)<br>C=C <sub>Ar</sub> (1596-1467)<br>$\nu\text{NH}_{2\text{asym}}$ (3400)<br>$\nu\text{NH}_{2\text{sym}}$ (3300) |
| H <sub>10</sub> | 3195                               | -----                                            | 3022                                              | 3379                               | C=O(1601)                                                                                                                 |

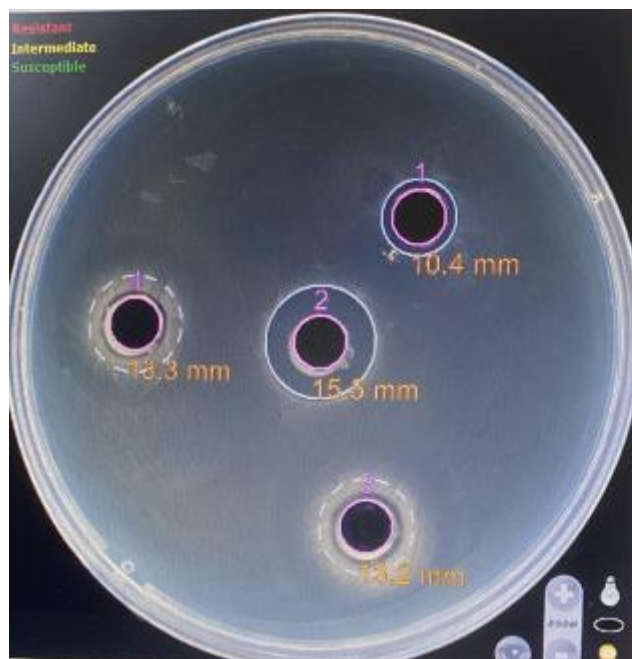
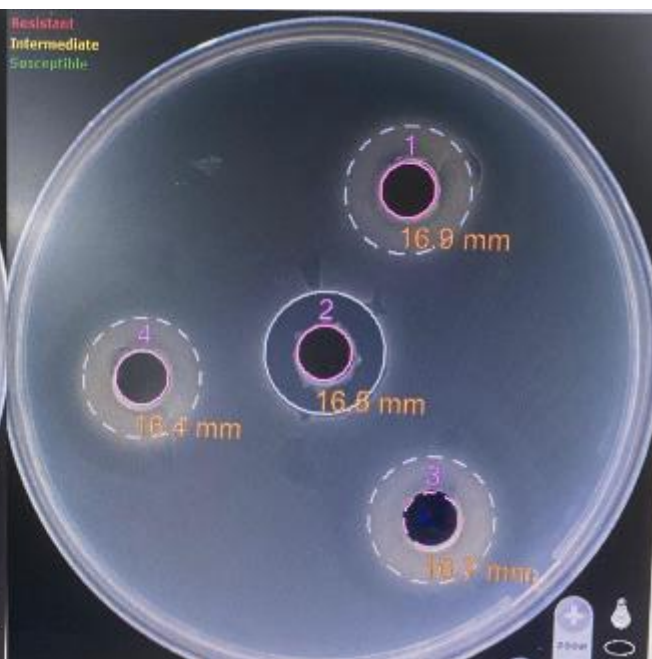
Table 3. XRD , FAM and FESEM data

| Comp.           | RMS<br>nm | PHR<br>nm | SA<br>$\mu\text{m}^2$ | TTR<br>nm | NFC<br>nm | $d_{\text{obs}}$<br>( $\text{\AA}$ ) | $\Delta d_{\text{obs}}$<br>( $\text{\AA}$ ) | $D_{\text{cal}}$ nm | $\Delta D_{\text{cal}}$<br>nm | $n_{\text{cal}}$ | $2\theta_{\text{obs}}$ |
|-----------------|-----------|-----------|-----------------------|-----------|-----------|--------------------------------------|---------------------------------------------|---------------------|-------------------------------|------------------|------------------------|
| H <sub>5</sub>  | 250       | 03.4      | 38.67                 | 41.34     | 03.19     | 3.36                                 | -4.15                                       | 19.55               | 9.64                          | 5.8              | 26.45                  |
| H <sub>6</sub>  | 121.2     | 12.02     | 35.18                 | 34.57     | 12.24     | 3.40                                 | -6.50                                       | 29.53               | 19.61                         | 8.66             | 26.15                  |
| H <sub>7</sub>  | 214.9     | 18.2      | 18.34                 | 149.27    | 21.54     | 3.38                                 | -4.13                                       | 28.95               | 19.04                         | 8.54             | 26.30                  |
| H <sub>9</sub>  | 186.8     | 22.4      | 24.68                 | 38.59     | 17.08     | 7.75                                 | 0.22-                                       | 6.51                | 3.39                          | 0.8              | 11.41                  |
| H <sub>10</sub> | 175.8     | 19.07     | 25.84                 | 33.07     | 21.98     | 3.27                                 | -4.24                                       | 1.76                | -8.14                         | 0.5              | 27.18                  |

RMS(Root Mean Square Roughness), PHR(Peak-to-Valley Height Roughness) ,SA(Surface Area) , TTR(Transition Texture Roughness), NFC(Nano fiber Cluster) ,  $d_{\text{obs}}$ (Observed Inter planar Spacing) ,  $D_{\text{cal}}$ (Calculated Crystallite Size) ,  $n_{\text{cal}}$ (Dislocation Density)

### Biological activity

Both Nano composites and heterocyclic compounds are defined as substances with diverse biological activities against fungi. In this research work, the biological activities of selected synthesized compounds have been studied for their action against the single species of fungus called *Candida albicans*. This particular species has been chosen for the experiment because of its significance in the medical field, causing various types of diseases, having different resistance levels against antibiotics. The antifungal activities of synthesized compounds have been assessed via the agar well diffusion technique in terms of zone of inhibition. The correlation between concentration and the efficiency of inhibition was investigated, and Table (5) below shows the correlation between concentration and inhibition efficiency. In Nano composites, an inverse relationship between concentration and inhibitory effect has been noted whereby an increase in the concentration reduces the effectiveness of the inhibitory effect. This is as a result of the increased viscosity of the Nano composites, which could cause aggregation and precipitation, hence reducing their diffusion through the agar as opposed to low concentrations.

Figure 13. Inhibition of Candida fungus by the H<sub>5</sub>Figure 14. Inhibition of Candida fungus by the H<sub>6</sub>

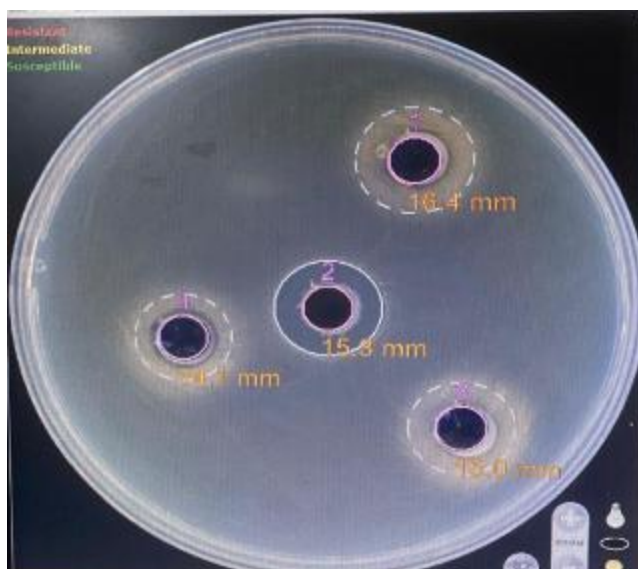


Figure 15. Inhibition of *Candida* fungus by the H<sub>7</sub>

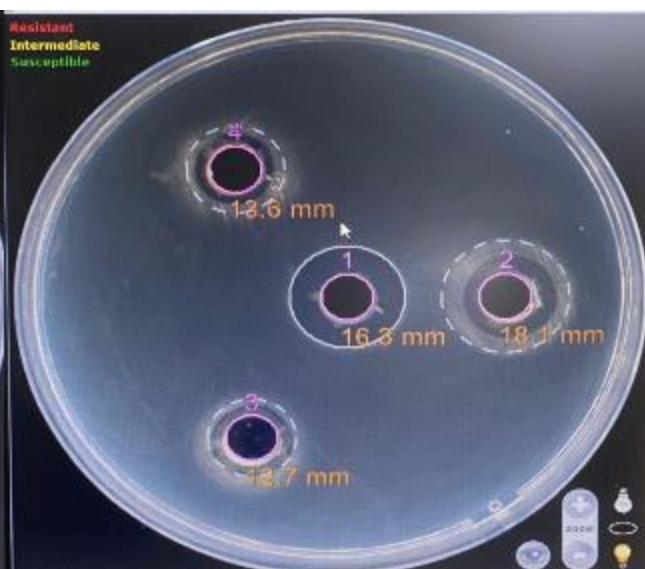


Figure 16. Inhibition of *Candida* fungus by the H<sub>9</sub>

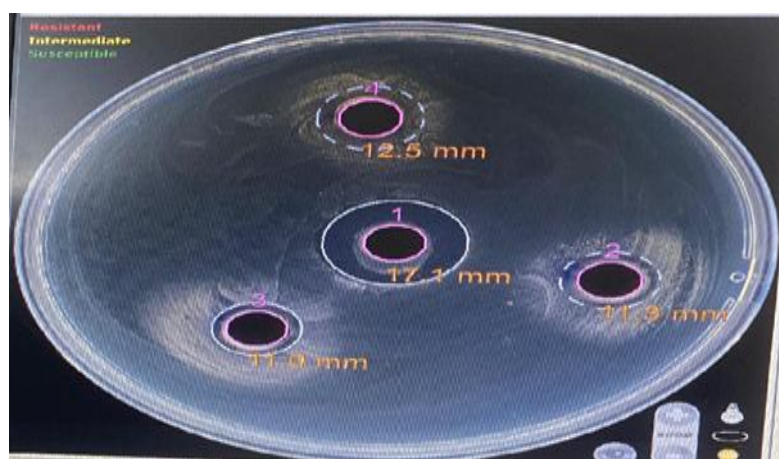


Figure 17. Inhibition of *Candida* fungus by the H<sub>10</sub>

Table 4. Shows the antifungal activity of the prepared compounds.

| <i>Candida</i>                        |                |                       |                         |
|---------------------------------------|----------------|-----------------------|-------------------------|
| Comp.                                 | Conc.<br>mg/ml | Zone inhibition<br>mm | Activity Level          |
| H <sub>5</sub><br>Gentamycin(control) | 25             | 10.4                  | Moderate Activity       |
|                                       | 50             | 13.2                  | Moderate / Intermediate |
|                                       | 100            | 13.3                  | Moderate / Intermediate |
|                                       |                | 15.5                  | Positive Control        |
| H <sub>6</sub><br>Gentamycin(control) | 25             | 16.9                  | High / Susceptible      |
|                                       | 50             | 16.7                  | High / Susceptible      |
|                                       | 100            | 16.4                  | High / Susceptible      |
|                                       |                | 16.5                  | Positive Control        |
| H <sub>7</sub><br>Gentamycin(control) | 25             | 16.4                  | High / Susceptible      |
|                                       | 50             | 16.0                  | High / Susceptible      |
|                                       | 100            | 14.1                  | Low                     |
|                                       |                | 15.3                  | Positive Control        |
| H <sub>9</sub>                        | 25             | 18.1                  | High / Susceptible      |
|                                       | 50             | 12.7                  | Moderate / Intermediate |
|                                       | 100            | 13.6                  | Moderate / Intermediate |
|                                       |                |                       |                         |

|                            |     |      |                  |
|----------------------------|-----|------|------------------|
| <b>Gentamycin(control)</b> |     | 16.3 | Positive Control |
|                            | 25  | 11.3 | Low              |
| <b>H<sub>10</sub></b>      | 50  | 11.0 | Low              |
|                            | 100 | 12.5 | Low              |
| <b>Gentamycin(control)</b> |     | 17.1 | Positive Control |

#### 4. Conclusion

FT-IR spectroscopy results demonstrated that GO synthesis through the modified Hummers' process was very successful as it resulted in the functionalization of the synthesized GO with aromatics and pharmaceuticals such as mefenamic acid and theophylline. The presence of some changes in the structures was observed, which included the appearance of new functional groups characterized by specific absorption bands of azomethine group {C=N} ( $1664\text{ cm}^{-1}$ ), amide group (C=O) at  $1689\text{ cm}^{-1}$ , and hydroxyl group (O-H) at ( $3404\text{--}3598\text{ cm}^{-1}$ ). It is an indication that the chemical reaction occurred successfully along with the hydrogen bonding and  $\pi$ - $\pi$  interaction. Changes in Crystal Structure and Partial Reduction (XRD) The XRD analysis confirmed the formation of the process of partial reduction of graphene oxide to form reduced graphene oxide (rGO) when synthesizing the Nano composites (especially H5 and H6). This was demonstrated by the total disappearance of the typical GO peak due to the presence of interlayers caused by the presence of oxygen-containing groups at the diffraction angle, and a new strong peak at  $2\theta = 26.15^\circ\text{--}26.45^\circ$  due to the (002) crystallographies plane. Such a situation is an indication of shrinking the distance between the layers (d-spacing) down to approximately  $0.34\text{ nm}$  and stacking of the graphene sheets together with the absence of the crystalline phase of the drug, which is an indication of its amorphization. From the FESEM and TEM images, it was clear that the Nano composites have the wrinkled and porous sheet-like morphology with extremely high surface area. The microstructural study found that there were sub-spherical nanoparticles evenly spread on the surface and inside the crevices of the graphene sheets. The particle size is very fine and varied from  $15.28\text{ nm}$  to  $39.99\text{ nm}$ . Such effective dispersion of particles avoided particle aggregation and made the Nano hybrid system physicochemically stable. The antifungal assessment results against *Candida albicans* demonstrated the supremacy of the prepared Nano composites, especially H6, H7, and H9. H9 had the maximum diameter of inhibition zone ( $18.1\text{ mm}$ ) at the dose level of  $25\text{ mg/mL}$ , surpassing the positive control (Gentamicin antibiotic), having  $16.3\text{ mm}$ . The most significant scientific result is the discovery of the inverse relationship between the concentration of the Nano composite and its inhibition efficiency: the inhibition zone diameters were reduced as the Nano composite concentration was increasing ( $25\text{--}100\text{ mg/mL}$ ). The main reason for such behavior lies in the increase of the viscosity of the Nano composite due to its high concentration that causes aggregation and precipitation of particles. As a consequence, it limits the physical mobility of the Nano composite and reduces its diffusion in the nutrient agar (Agar diffusion failure).

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