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Synthesis, Characterization and Antibacterial Activity of Kaolin/Gum Arabic Nanocomposite on Escherichia Coli and Pseudomonas Aeruginosa

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ABSTRACT

The research and development of Nanocomposites has increased nowadays due to their incredible applications. In this project, the synthesis of Kaolin/gum Arabic nanocomposite was carried out by using the method of solution mixing. The resulted nanocomposite was characterized by using FTIR, SEM and Ultraviolet-visible absorption spectroscopy. Antibacterial activity of the Kaolin, Gum Arabic and synthesized nanocomposite on E.coli and Pseudomonas aeruginosa were carriedout by agar well diffusion method. The Kaolin was observed to have no antibacterial activity, the Gum Arabic and Kaolin/Gum Arabic nanocomposites have remarkable activities on E. coli and Pseudomonas aeruginosa bacteria. The antibacterial activity of Gum Arabic extract on E. coli were found to be 29mm, 18m and 12mm at 100, 50 and 25µg/L concentrations respectively and that of the Kaolin/Gum Arabic nanocomposite were; 16mm, 11mm and 4mm at 100, 50 and 25µ/L concentrations respectively. The antibacterial studies of Gum Arabic extract at various concentrations 100µL, 50µL and 25µg/L were found to be 38mm, 25mm and 14mm respectively for Pseudomonas aeruginosa. Also, the antibacterial activity of Kaolin/Gum Arabic nanocomposite at various concentrations 100µg/L, 50µg/L and 25µg/L were found to be 27mm, 15mm and 9mm respectively Pseudomonas aeruginosa. The result suggests that the Kaolin/Gum Arabic Nanocomposites can serve as a potential antibacterial agent.

Keywords: *Kaolin, Gum Arabic, Nanocomposite, Antibacteria*

INTRODUCTION

Polymer composites (PC's) consist of different types of short or continuous fibers bound together by an organic polymer matrix. These are often divided into two categories; reinforced plastics and advanced composites. This difference is based on the level of the mechanical properties, for instance, strength and stiffness. Some advantages of these compounds are their lightweight coupled with high stiffness along the direction of the reinforcement. They are constituted of two phases: the continuous phase called matrix, and the dispersed phase that is mainly called as the reinforcing phase. In general, nanocomposites consist of matrices of different materials, which can be polymer, metal or ceramic, and to which is added the charge, e.g., multiwalled carbon nanotubes, activated carbon, reduced graphene oxide, natural clays, graphene-like materials etc., that gives new properties to the material, which helps in a specifc application. Among these types, polymeric ones have proven to be the most versatile for their application in several research felds like engineering, building, ptoelectronics, medicine, and environmental remediation as well as antimicrobial activity test. [1-3]. Matrix consist of polymers such as thermostable, thermoplastic, general use polymer [4-6], engineering plastic [7-9], conductive polymers[10, 11] and biodegradable polymers[12]. Whereas reinforced phase or filler consist of the following examples such as carbon nanotube, carbon nanofiber, graphene, nanocrystalline metals,, modified fiber, nanoscale inorganic fillers and clay. Thus, clay as a filler produce advantages in the mechanical properties for the modification of polymeric system [13-15].

Infectious diseases show an important health problem and represent one of the main causes of mortality worldwide, due to the indiscriminate use of antibiotics and incidence of multiple antibiotic resistances in human pathogens. Due to report of increasing developments of drug resistance in human pathogen, it is necessary to search for new agents which are better and without side effect for treating diseases especially in developing countries. Antimicrobial activity of Acacia was reported to be concentrated either in leaves or bark and their extracts have been reported inhibitory against Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Salmonella Typhi, Bacillus subtilis, B. creus, Shigella sonnei and even against Candida albicans, C. glabrata and Aspergillus niger and Rhizoctonia solani [16-21]. The antimicrobial activity has been chiefly detected in methanolic extracts rather than in aqueous extract [20]. Also, Kaolin base nanocomposites[22] and gum Arabic ethanolic extracts were found be effective against bacteria isolates[23].

Aim of the present study was designed to evaluate the antibacterial potential of Kaolin, Gum Arabic and Kaolin/Gum Arabic Nanocomposite against E.coli and Pseudomonas aeruginosa bacteria isolates.

MATERIALS AND METHOD

List of Apparatus/Intruments

The apparatus that were used in this experiment consist of; motar and pestle, siever, beakers, conical flasks, test tubes, measuring cylinder, whatman filter paper, watch glass, oven, weighing balance, funnels, hot plate, autoclave, petri dish, incubator, refridgerator, laminar flow cabinet, cotton wool, aluminium foil paper, candle, matches, universal container, cork borer, metre rule, masking tape, spatula, glass rod stirrer, syringes, micropipette, wire loop, Ultraviolet –Visible spectrophotometer, SEM, and FTIR.

List of Reagents

The reagents that were used are as follows; Ethanol, Hydrochloric acid (HCl), Tetraoxosulphate (VI) acid (H_2SO_4), barium chloride ($Bacl_2$), Normal saline, nutrient Agar, and Muller Hinton agar.

Sampling Collection

Gum Arabic was obtained from Gombe Old Market in Gombe State, and Kaolin was obtained from the area of Arawa in Gombe, Gome State. The bacteria isolate of Escherichia coli (E. Coli) and Pseudomonas aeruginosa were obtained in the Microbiology laboratory of Gombe State University Tudun Wada, Gombe, Gombe State, Nigeria.

Purification of Kaolin

Kaolin was sieved using 105 mesh size siever and 322g of Kaolin was soaked in 1000ml of 1m HCL. The solution was stirred and allowed to stand for an hour after which the supernatant was gently discarded leaving only the pure kaolin. The purified kaolin was then dried in an oven at the temperature of 105^{o}C for 3 hours and kept for analysis [24].

Preparation of Gum Arabic Extract

Gum Arabic Extract was prepared using the method that was employed by [25, 26]. Gum Arabic was pounded and sieved with 105 mesh size sieve. 250g of powdered gum Arabic was mixed in a 1000ml conical flask with 500ml of 99% pure ethanol and the flask was kept overnight at 25^{o}C. Thereafter, all the contents of the flask were filtered through whatman filter paper to recover the filtrate. The filtrate was transferred to a sterile conical flask and kept for analysis.

Synthesis of Kaolin/Gum Arabic Nanocomposite

The synthesis of the Kaolin/Gum Arabic Nanocomposite was carried out by the method of solution mixing. 161g of purified Kaolin was weight and soaked in 500ml of Gum Arabic ethanolic extract in a beaker. It was then stirred and aged for 24 hours (One day). The mixture was then filtered separating the solid content and dried in an oven at a temperature of 105^{o}C for 3 hours after which it was kept for analysis [27].

Anti-Bacterial Studies

Bacterial Culture

20g of nutrient Agar was dissolved in 1000ml of dionized water, the mixture was heated on a hot plate and then autoclaved for 15 minutes at the temperature of 121^{o}C. The mixture was allowed to cooled and then poured on Petri dishes. After the nutrient agar has solidified inside the petri dish, pure isolate of the bacteria Escherichia coli (E. Coli) and Pseudomonas aeruginosa was each grown on the different agar plates at 37^{o}C for 24 hours in an incubator.

Preparation of 0.5 McFarland Standard

1g of Barium chloride ($BaCl_2$) was dissolved in 100ml of distilled water, and 1ml of concentrated (VI) acid (H_2SO_4) was dissolved in 99ml of distilled water. Then 9.9ml of the $BaCl_2$ solution was taken in a test tube and 0.1ml of the solution of H_2SO_4 was added to it to give 10ml of McFarland standard.

Mueller Hinton agar was prepared by dissolving 34g of it in 1000ml of deionized water; the mixture was heated and autoclaved at 121°C for 15 minutes. It was then cooled and poured on petri dishes. 1g of Kaolin and the Kaolin/Gum Arabic Nanocomposite was each dissolved in 5ml of distilled water to form Kaolin and Kaolin/Gum Arabic Nanocomposite suspension respectively.

The gum Arabic ethanolic extract, Kaolin and Kaolin/Gum Arabic Nanocomposite suspension were used for evaluation of antibacterial activity by the agar well diffusion method. In this method, after growing bacteria on agar plates (culturing), the bacterial culture was transferred in to normal saline under aseptic conditions, and it was used as the inoculums for performing on agar well diffusion assay. The inoculums were spread on to the agar plates and were allowed to dry. Then, 6mm wells were made with a sterile cork borer in the inoculated agar plates, and various concentrations 25, 50 and 100 micro litre of each extract and suspension were propelled directly into the plates. The plates were allowed to stand for 1 hour at room temperature for diffusion of the extracts into agar and incubated at 37°C for 24 hours. The antibacterial activity was indicated by an inhibition zone surrounding the well containing the extract and suspension, and it were recorded if the zone was greater than 6mm. The experiments were performed in triplicate and the mean values of the diameter of inhibition zone were calculated.

RESULTS AND DISCUSSIONS

Characterization

In this study, kaolin, gum Arabic and kaolin/gum Arabic nanocomposite were characterized using FTIR to detect the functional groups present, Ultraviolet-Visible spectrophotometer and Scanning Electron Microscopy.

FTIR Results

In the FTIR analysis of Kaolin, the bands were found at 3696.45cm^{-1}, 3620.13cm^{-1} and 3449.40cm^{-1} corresponds to the inner and outer structural hydroxyl Al-OH stretching with medium strength and symmetric O-H stretching respectively. Band at 2924.19cm^{-1} and 2853.42cm^{-1} belongs to C-H. There was also O-H bending vibration mode at 1615.16cm^{-1}, Figure 1 and Table 1.

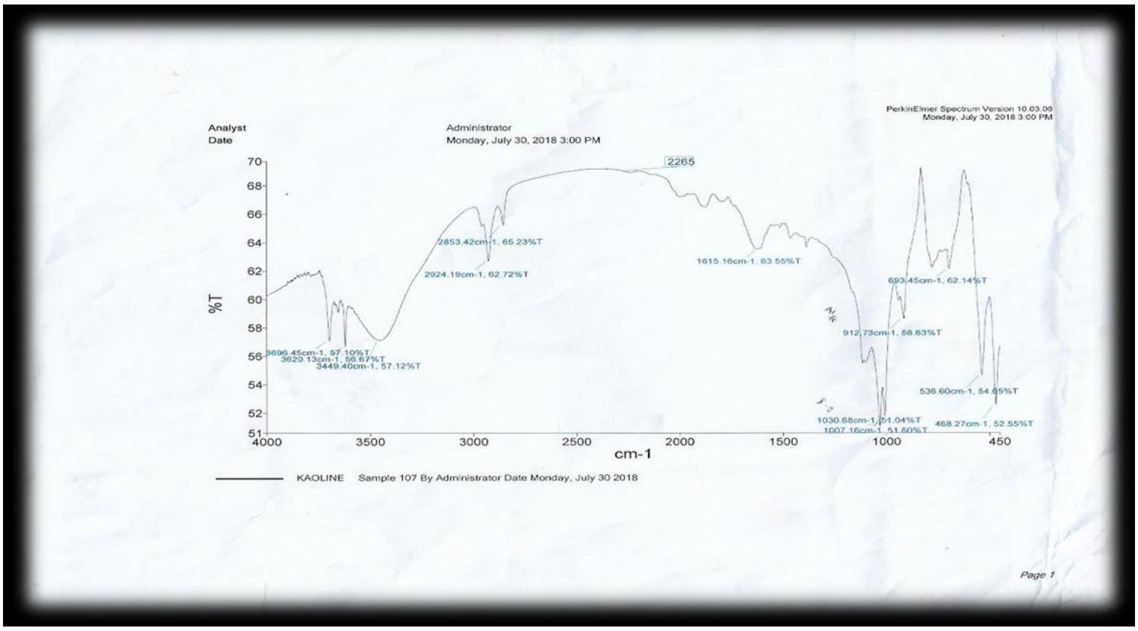

Figure1. *FTIR spectrum for Kaolin.*

The IR spectrum for gum Arabic showed a typical characteristic of absorption bands for common polysaccharide. The FTIR showed wide stretch of O-H group at 3437.50cm-1, C=C at 1633.33cm-1 and C-H at 2924.40cm-1, Figure 2 and Table1.

FTIR of Kaolin/Gum Arabic Nanocomposite in comparism to Kaolin and Gum Arabic also showed a peak medium in stretching at 3695.96cm-1 which correspond to Al-OH stretching, another band was seen at 3439.91cm-1 which is the O-H stretching, C-H stretching vibration also appeared at 2924.50cm-1 and a broad Si-OH stretching mode at 1871cm-1, O-H bending vibration can be seen at 1610.05cm-1, Figure 3 and Table 1. These results represent the major bands in the FTIR spectrum for Kaolin, Gum Arabic and Kaolin/Gum Arabic Nanocomposite. The only exception is the stretching band at 1633.33cm-1 which correspond to C=C found in Gum Arabic.

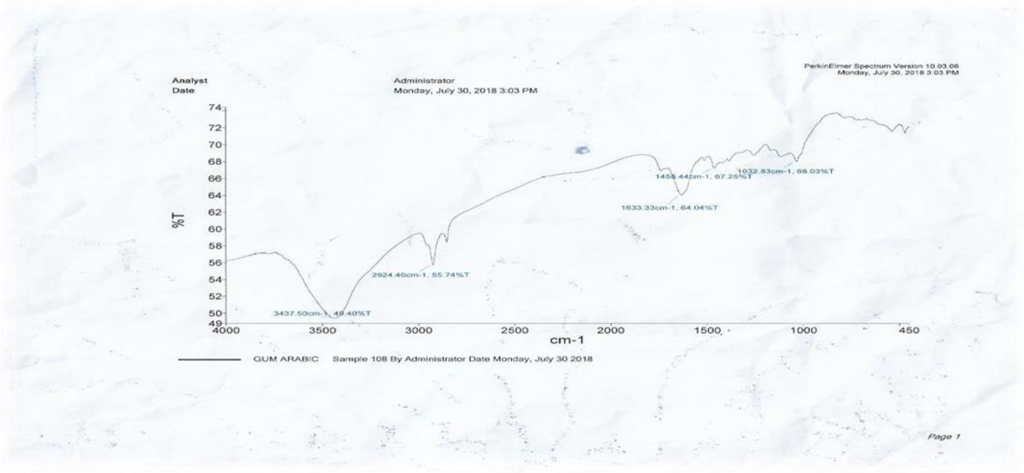

Figure2. *FTIR spectrum for Gum Arabic*

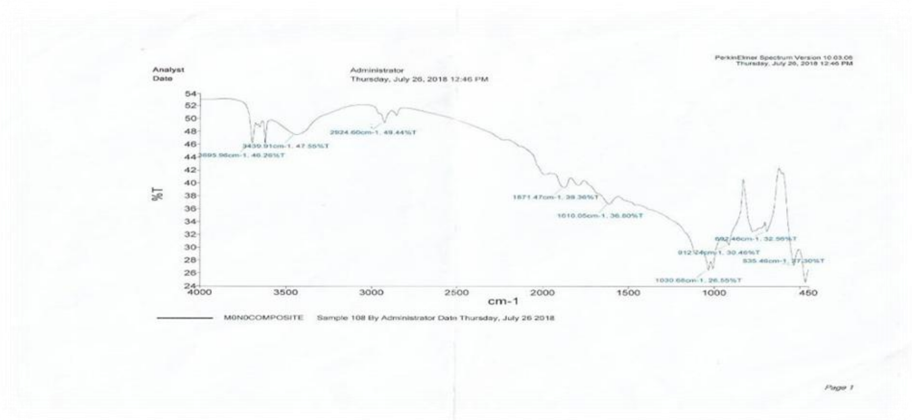

Figure3. *FTIR spectrum for Kaolin/Gum Arabic Nanocomposite*

Table1. *Summary of FTIR spectroscopy for the Kaolin/Gum Arabic Nanocomposite, Kaolin and Gum Arabic*

Sample	Absorption band	Functional group
Kaolin/Gum Arabic Nanocomposite	$3695.96cm^{-1}$	Al-OH
	$3439.91cm^{-1}$	O-H
	$2924.50cm^{-1}$	C-H
	$1871.47cm^{-1}$	Si-OH
Kaolin	$3696.45cm^{-1}$	Al-OH (inner)
	$3620.13cm^{-1}$	Al-OH (outer)
	$3449.40cm^{-1}$	O-H (symmetric)
	$2924.19cm^{-1}$	C-H
	$2853.42cm^{-1}$	C-H
	$1615.16cm^{-1}$	O-H (bending vibration)
Gum Arabic	$3437.50cm^{-1}$	O-H
	$2924.40cm^{-1}$	C-H
	$1633.33cm^{-1}$	C=C

Ultraviolet-Visible Spectroscopy

The Ultraviolet-visible spectrophotometer was used to measure the optical absorption of the Kaolin/Gum Arabic Nanocomposite with the highest absorbance at 1.567 which has the maximum wavelength (λmax) at 203.5nm as it can be seen in Figure 4 below.

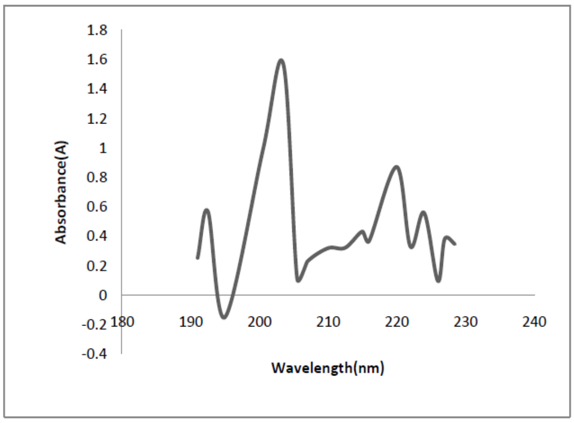

Figure4. *Ultraviolet-visible Spectrum for Kaolin/Gum Arabic Nanocomposite*

Scanning Electron Microscopy Analysis

The SEM analysis of the Kaolin/gum Arabic Nanocomposite was used to investigate the surface morphology and structure of the Nanocomposite. The SEM images of the Nanocomposite are displayed below at different magnifications, Figure 5 and 6. The SEM images of the Nanocomposite have rough appearance and agglomeration nanocomposite. The SEM images of the studied sample proved matrix consisting of microsized Kaolinite particles having layered structure.

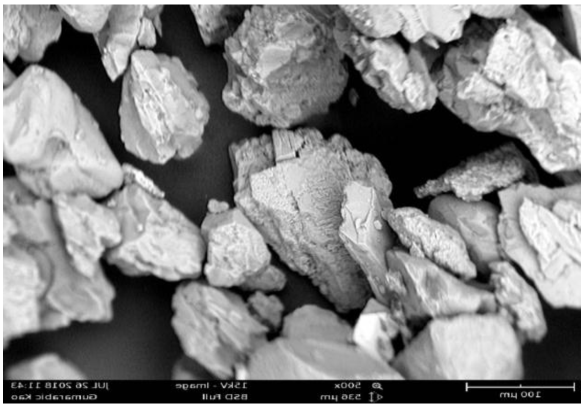

Figure5. *SEM image A*

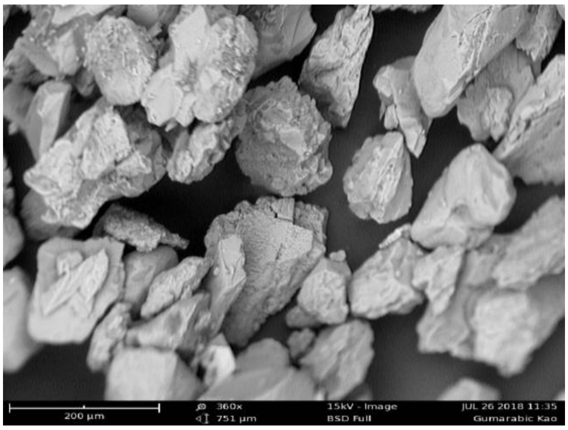

Figure6. *SEM image B*

Table3. *Results for antibacterial analysis*

Tested Bacteria	Antibacterial Agent		Zone of inhibition (mm)*		
		25µg/L	50µg/L	100µg/L	Mean
	Kaolin	ND	ND	ND	
	Gum Arabic	29	18	12	20
Escherichia coli	Kaolin/Gum Arabic nanocomposite	16	11	4	10
	Kaolin	ND	ND	ND	
	Gum Arabic	38	25	14	26
Pseudomonas aeruginosa	Kaolin/Gum Arabic nanocomposite	27	15	9	17

*ND; Not Detected and *; Each value in the table was obtained by calculating the mean value of three experiments*

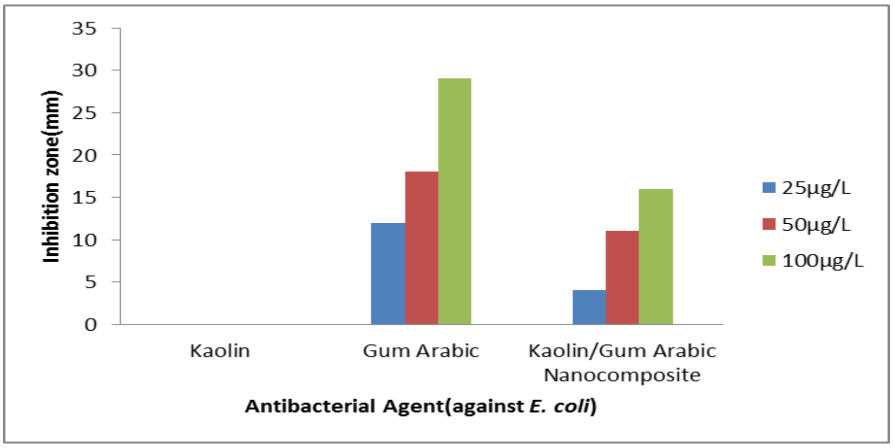

Figure7. *Antibacterial activity of Kaolin, Gum Arabic and Kaolin/Gum Arabic Nanocomposite on Escherichia coli*

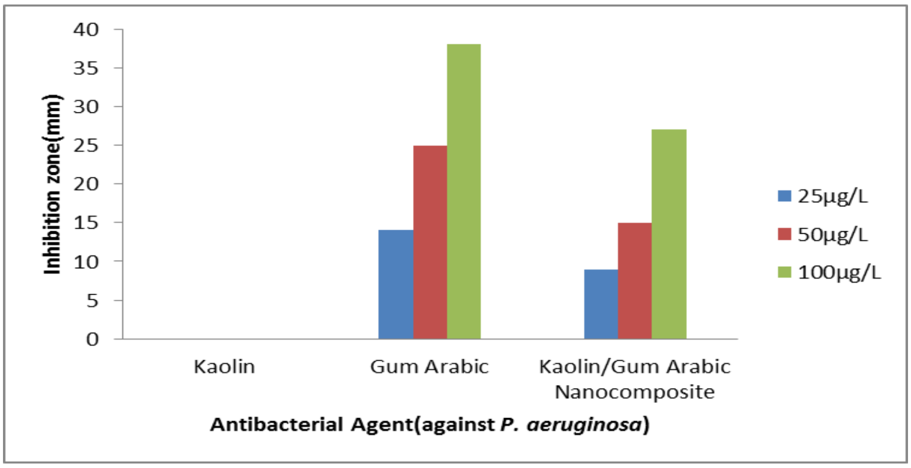

Figure8. *Antibacterial activity of Kaolin, Gum Arabic and Kaolin/Gum Arabic Nanocomposite on Pseudonomas aeruginosa*

Conclusion

This research has focused on the synthesis, characterization and antibacterial activity of Kaolin/Gum Arabic Nanocomposites. The Nanocomposite was characterized using various analytical techniques; FTIR, UV-Visible Spectroscopy and SEM. The result on the SEM analysis of Kaolin/Gum Arabic Nanocomposites showed that the Nanocomposites have rough surface of the layered structure of kaolinite. The FTIR analysis of the Gum Arabic and Kaolin/Gum Arabic Nanocomposite has a significant difference only in the absorption band at 1633cm-1 due to C=C found in Gum Arabic but not in the Nanocomposite. The Ultraviolet-visible analysis showed that the Nanocomposite has its highest absorption at 1.567, with its corresponding wavelength (λmax) at 203.5nm. From the antibacterial screening, it could be concluded that Kaolin has no antibacterial activity on the Escherichia coli and Pseudomonas aeruginosa bacteria. Yet, the Gum Arabic and the Kaolin/Gum Arabic Nanocomposite proved to be a potent antibacterial agent for both Escherichia coli and Pseudomonas aeruginosa bacteria. The activity of Gum Arabic extract against Pseudomonas aeruginosa bacteria was found to be 38mm, 25mm and 14mm at various concentrations (100µL, 50µL and 25µL) respectively. Whereas for the Kaolin/Gum Arabic Nanocomposites against Pseudomonas aeruginosa, the inhibition zone were found to be 27mm, 15mm, and 9mm at various concentrations (100µL, 50µL and 25µL) respectively. The antibacterial assay using Escherichia coli (E.Coli), proved that the nanocomposite do have antibacterial potency which is from the gum Arabic extract. The inhibition zones by the Gum Arabic extract against Escherichia coli were; 29mm, 18mm and 12mm at 100, 50 and 25µl concentrations respectively while for the Kaolin/Gum Arabic Nanocomposite were; 16.33mm, 11.33mm, and 3.7mm at the same concentrations. The result suggests that the Kaolin/Gum Arabic Nanocomposites can serve as a potential antibacterial agent.

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