ISSN: 0067-2904

Kaolin/ Arabic Gum Nanocomposite as an Antifungal Agent Against *Aspergillus flavus and Saccharomyces Cerevisiae*

Zaccheus Shehu*[1], Danbature Wilson Lamayi[1], Maisanda Adunbe Sabo[1], and Sani Ibrahim Aliyu[2]
[1] Chemistry Department, Faculty of Science Gombe State University, Nigeria
[2]Microbiology Laboratory, Gombe State University, Nigeria

Received: 11 /3 / 2019 Accepted: 18 / 6/2019

Abstract
Kaolin/ Arabic Gum nanocomposite was cheaply synthesized from Kaolin and Arabic Gum. The Kaolin/ Arabic Gum nanocomposite suspension, Arabic Gum extracts and Kaolin suspension were applied as antifungal agents. The antifungal activity was tested using the agar well diffusion method, whereby wells were made on the petri dishes with a cork borer of 6 mm diameter in size.Various concentrations (150 µg/L, 200 µg/L, and 250 µg/L) of Arabic Gum ethanol extracts, Arabic Gum/ Kaolin nanocomposite, and Kaolin were propelled into the wells with the help of a micropipette and the petri dishes were allowed to stand for 30 minutes to ensure proper diffusion before being incubated at 37^{o}C. The results showed that the synthesized Kaolin/ Arabic Gum nanocomposite and Arabic Gum possess significant antifungal activities against *Aspergillusflavus* and *Saccharomyces cerevisiae*. No antifungal activity was detected for Kaolin against *Aspergillusflavus* and *Saccharomyces cerevisiae*. From the results obtained, it could be concluded that the synthesized Kaolin/ Arabic Gum nanocomposite and Arabic Gum possess significant antifungal activity against *Aspergillusflavus* and *Saccharomyces cerevisiae*.

Keywords: Kaolin/ Arabic Gum nanocomposite, *Saccharomyces cerevisiae*, *Aspergillusflavus*, Antifungal, Gum Arabic

1.0 INTRODUCTION

Nanoparticles and nanocomposites are the cornerstone for the emerging nano science and technology that is heading towards getting things smaller [1]. The properties of nanomaterials differ from their bulk material counterparts, and materials can self-assemble spontaneously into ordered structures [2]. Nanoscience is emerging as a powerful tool in developing new approaches in the field of designing new antifungal and antimicrobial drugs [3]. This is due to the fact that it is difficult to control the growth of fungi because resistance to many antifungal agents [4]. A wide range of work on antimicrobial activities of nanomaterialswas performed globally by different authors, but the antimicrobial activities of silver nanoparticles synthesized using different methods were studied extensively [1, 5-9]. The antimicrobial potential of semiconductors-based nanoparticles such as zinc sulphide [10], Zinc oxides [4, 11] as well as copper nanoparticles [12] were studied. The antibacterial effect of $NiFe_2O_4$@TiO_2/Ptnanocomposite on *E.Coli* bacteria was found to be effective [13]. Various natural polymers and their composites such as Silver-Kappa-Carrageenan [14], Chitosan silver nanocomposite [15], Silver/Chitosan nanoformulation[16], Collagen based nanocomposites [3], natural edible Gums extract[17, 18], Arabic Gum [19, 20] as well as porphyrin-polymer

*Email: zaccheusshehu@gmail.com

nanocompartments [21] were evaluated and found to inhibit microorganism growth. The antimicrobial nanocomposites based on kaonite were investigated by some authors [22-24].

In our previous work we tested the antibacterial activity of synthesized Kaolin/ Arabic Gum nanocomposite [25]. Therefore, this study aimed at testing the antifungal potential of Kaoli/ Arabic Gumnanocomposite, Kaolin, as well as Arabic Gum extracts on *Sccharomycescerevisiae* and *Aspergillusflavus*.

2.0MATERIALS and METHOD

2.1 Apparatus/ Instruments/ Reagents

The apparatus that were used in this experiment consisted ofmotar and pestle, sieve, micropipette, wire loop, ethanol, hydrochloric acid (HCl), tetraoxosulphate (VI) acid (H_2SO_4), barium chloride ($BaCl_2$), normal saline, nutrient agar, and Muller Hinton agar.

2.2 Sample Collection

Arabic Gum was obtained from Gombe Old Market in Gombe State, kaolin was obtained from Arawa area in Gombe, Gombe State and the fungi isolates of *Aspergillusflavus*and *Saccharomyces cerevisiae* were available in the Microbiology Laboratory of Gombe State University Tudun Wada, Gombe, Gombe State.

2.3 Preparation of Solutions

Preparation of ~1M hydrochloric acid (HCl): ~1M HCl was performed by adding 83ml of HCl stock solution to 1000ml of deionized water in accordance with the standard which is 8.3ml of HCl stock solution in 100ml of water.

Kaolin was sieved using 105 mesh size sieve 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.

Gum Arabic Extract was prepared using the method that was employed by Singh *et al*. [18]. Arabic Gum was pounded and sieved with 105 mesh size sieve, 250g of powdered Arabic Gum 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 papers to recover the filtrate; the filtrate was transferred to a sterile conical flask and kept for analysis.

2.4 Synthesis of KGA Nanocomposite

The synthesis of Kaolin/ Arabic Gum (KGA) nanocomposite and its characterizations by FTIR, UV/ Visible spectrophotometer and SEMwere carried out according to the methodology previously described by our group[25].

2.5 Preparation of Culture Media

To culture the fungi, 45g of malt agar was dissolved in 1000 ml of distilled water, mixed thoroughly, and heated till a clear solution was formed. The solution was then autoclaved at 118^{o}C for 15 minutes and allowed to cool. 250 mg of chlorophenecol was added to the agar solution to avoid bacteria interference.It was then poured on petri dishes and allowed to solidify. Afterwards, fungi species of *Aspergillusflavus*and *Saccharomyces cerevisiae*were separately cultured on petri dishes and incubated for 48 hours. After the incubation period, fungi growth was observed inside the petri dishes.

2.5.1 Sub Culture Media

The fungi were sub cultured in potato dextrose agar (PDA) by dissolving 39g of PDA in 1000ml of distilled water. The agar was heated to dissolve till it became a clear solution, which was autoclaved at 105^{o}C for 15 minutes, then cooled and poured on petri dishes. The cultured *Aspergillusflavus*and *Saccharomyces cerevisiae*were separately sub cultured on the PDA inside different petri dishes.

2.6 Antifungal Activity

The anifungal activity was tested using agar well diffusion method. Wells were made on petri dishes with cork borer (6 mm diameter), into which various concentrations of Arabic Gum ethanol extract, Kaolin/ Arabic Gum nanocomposite and Kaolin (150µg/L, 200µg/L, 250µg/L, respectively) were propelled with the help of a micropipette. The petri dishes were allowed to stand for 30 minutes to ensure proper diffusion before being incubated at 37^{o}C. For positive control, 150µl of Ketoconazole solution was propelled into the 6 mm wellsfilled with potato dextrose agar (PDA),in which fungi species of *Aspergillusflavus* and *Sccharomycescerevisiae* were sub cultured. After 48 hours, zones of inhibition were seen near the wells and were measured and recorded.

3.0 RESULTS AND DISCUSSION

In this work, the antifungal activity of the Arabic Gum extract, Kaolin and the Kaolin/ Arabic Gum nanocomposite were tested against *Aspergillusflavus*and *Saccharomyces cerevisiae*. As the concentrations of Arabic Gum extracts and Kaolin/ Arabic Gum nanocomposite suspensionwere increased from 150 to 250 µg/ L, the inhibition zone diametersof *Saccharomyces cerevisiae*were increased from 19 to 21.5 mm and 11 to 14.5 mm, respectively. For *Aspergillusflavus*, as the concentration of Arabic Gum extracts and Kaolin/ Arabic Gum nanocompositesuspension wereincreased from 150 to 250 µg/L, the inhibition zone diameters were increased from 21.5 to 25.0 mm and 10 to 14 mm, respectively (Table 1). This phenomenon of the increase in concentration of antifungal agents with increase in the diameter of the inhibition zone was reported by some authors [14-25]. No antifungal activity detected for Kaolin against *Aspergillusflavus* and *Saccharomyces cerevisiae*. Some authors also reported that Kaolin has no antimicrobial activity [22-25]. Among the three antifungal agents, Arabic Gum extract had the highest inhibition against the fungi, followed by Kaolin/ Arabic Gum nanocomposite. The inhibition zone of Ketoconazole (control) against *Aspergillusflavus* and *Saccharomyces cerevisiae* was higher than that of the other three antifungal agents. This was also observed in the studiesof Vijay *et al*., and Kaur*et al*., [12, 16] where the standard antibiotic inhibition tends to be higher than the tested antifungal agents. The antifungal activities of Arabic Gum extracts, and Kaolin/ Arabic Gum nanocomposite were found to be higher on *Aspergillusflavus* than *Sccharomycescerevisiae*(Figure 1). It is quite interesting to know that the most popular studied antimicrobial agents (e.g., Chitosan, Silver nanoparticles and Chitosan/Silver nanocomposite) possessed inhibition zone of 10.66, 10.0, and 19.66 mm respectively for *A. flavus* [12]. Thus, this study proved to demonstrate better antifungal activity.

Table 1-Results for the antifungal activity

Antifungal agents	*S.cerevisiae* inhibition zone(mm)				*A. flavus* inhibition zone(mm)			
	150 µg/L	200 µg/L	250 µg/L	mean	150 µg/L	200 µg/L	250 µg/L	Mean
Gum Arabic	19.0	20.0	21.5	20.2	21.5	24.5	25.0	23.7
Kaolin/Gum Arabic nanocomposite	11	12	14.5	12.5	10	12	14	12.0
Kaolin	ND	ND	ND	ND	ND	ND	ND	ND
Ketoconazole(control)			33				38	

Note: ND means not detected.

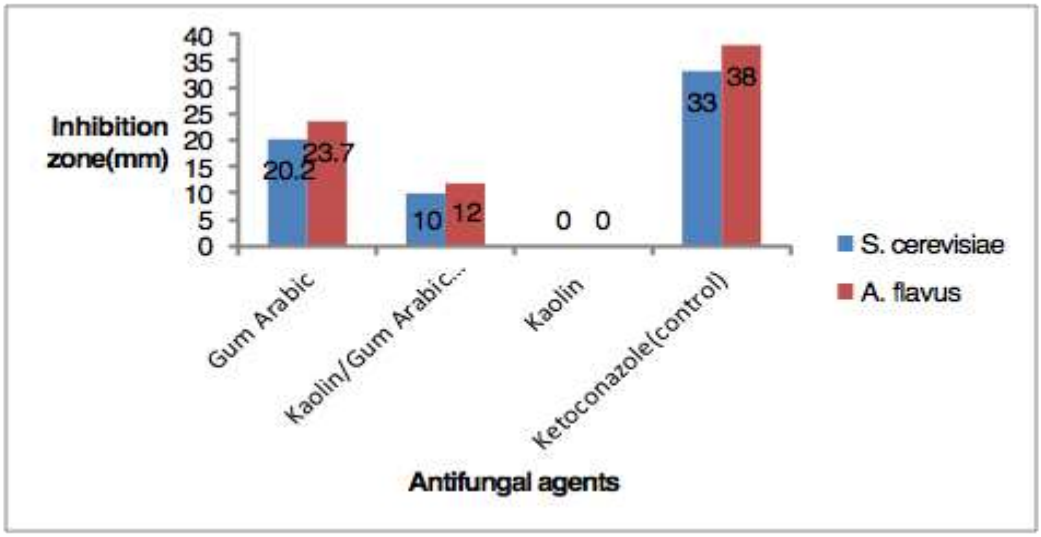

Figure 1- Antifungal activity of Arabic Gum extracts, Kaolin/ Arabic Gum nanocomposite, Kaolin and Ketoconazole (control) against *A. flavus* and *S. cerevisiae*

Conclusions

Kaolin/ Arabic Gum nanocomposite was cheaply synthesized from Kaolin and Arabic Gum. The Kaolin/ Arabic Gum nanocomposite suspension, Arabic Gum extracts and Kaolin suspension were applied as antifungal agents. The results showed that the synthesized Kaolin/ Arabic Gum nanocomposite and Arabic Gum possess significant antifungal activities against *Aspergillusflavus* and *Saccharomyces cerevisiae*.

Conflict of Interest

No conflict of interest.

References

1. Thomas B, Prasad A. A. and Vithiya, S. M. **2018**. Evaluation of Antioxidant, Antibacterial and Photo Catalytic Effect of Silver Nanoparticles from Methanolic Extract of Coleus Vettiveroids–an Endemic Species. *J. Nanostruct.* **8**(2):179-190. DOI:10.22052/JNS.2018.02.008
2. Wilson Lamayi D, Shehu Z. and Solomon Kwarson P. **2018**. Aqueous Phase Removal of Fluoride as Fluorosis agent Using Montmorillonite Clay as a Natural Nanoadsorbent. *Nanochem Res*. **3**(2): 219-226. DOI: 10.22036/ncr.2018.02.012
3. Stoyanova DS, Ivanova IA, Staneva A, Alby-Kaya M. and Vladkova, TG. **2016**. Antifungal potential of some collagen-based nanocomposites Against Candida lusitaniae. *NanosciTechnol*. **3**(1): 1-7.
4. Neeran Obied, J. **2015**.Antifungal Activity of Zinc Oxide Nanoparticles on AspergillusFumigatus Fungus &Candida Albicans Yeast, *J. of Nat Sciences Res*, **5**(4): 23-27.
5. Komal, R. and Kashyap S. **2018**. Characterization and Assessment of Antimicrobial Activity and Potential of Heavy Metal ion Detectionof Silver Nanoparticles synthesized from Actinidiadeliciosa paste using double distilled water and 70% ethanol as solvent. *J. Nanostruct.* **8**(4): 332-342. DOI: 10.22052/JNS.2018.04.002
6. Francis, S. Koshy, E. and Mathew, B. **2018**. Microwave Aided Synthesis of Silver and Gold Nanoparticles and their Antioxidant, Antimicrobial and Catalytic Potentials. *J Nanostruct*. **8**(1): 55-66. DOI: 10.22052/JNS.2018.01.007
7. Singh, J., Singh, N., Rathi, A., Kukkar, D. and Rawat, M. **2017**. Facile Approach to Synthesize and Characterization of Silver Nanoparticles by Using Mulberry Leaves Extract in Aqueous Medium and its Application in Antimicrobial Activity. *J.Nanostruct*, **7**(2): 134-140. DOI: 10.22052/jns.2017.02.007
8. Mahdavi-Ourtakand M., Jafari P. and Safaeijavan, R. **2017**.Antibacterial Activity of Biosynthesized Silver Nanoparticles from Fruit Extracts of BuniumpersicumBoiss, *Int. J. Bio-Inorg. Hybr. Nanomater*. **6**(4): 245-251.

9. Banerjee, P. and Nath, D. **2015**. A Phytochemical Approach to Synthesize Silver Nanoparticles for Non-Toxic Biomedical Application and Study on their Antibacterial Efficacy. *NanosciTechnol.* **2** (1): 1-14.

10. Mani S. K, Manickam S, Muthusamy V. and Tangaraj R. **2018**. Antimicrobial Activity and Photocatalytic Degradation Properties of Zinc Sulfde Nanoparticles Synthesized by Using Plant Extracts. *J. Nanostruct,* **8**(2):107-118. DOI: 10.22052/JNS.2018.02.001

11. Auyeung A., Casillas-Santana M.A., Martı ´nezCastaño´n G.A., Slavin Y.N., Zhao W., Asnis J., Ha¨feli U.O., Bach H.**2017**.Effective Control of Molds Using a Combination of Nanoparticles. *PLoS ONE*, **12**(1): 1-13. DOI:10.1371/journal.pone.0169940

12. Vijay Kumar P. P. N., Shameem U, Kollu P, Kalyani R. L. and Pammi S. V. N. **2015** Green Synthesis of Copper Oxide Nanoparticles Using Aloe vera Leaf Extract and Its Antibacterial Activity Against Fish Bacterial Pathogens.*BioNanoSci.,*; **5**(2): 65-68, DOI 10.1007/s12668-015-0171-z

13. Nabiyouni G. and Ghanbari, D.**2018**. Simple Preparation of Magnetic, Antibacterial and Photo-Catalyst $NiFe_2O_4$@TiO2/PtNanocomposites. *J.Nanostruct,* **8**(4): 408-416. DOI: 10.22052/ JNS. 2018.04.011

14. Hosseinzadeh, H. **2016**. Microwave-Assisted Synthesis of Kappa-Carrageenan Beads Containing Silver Nanoparticles with Dye Adsorption and Antibacterial Properties. *J. Nanostruct.*, **6**(2): 132-139. DOI: 10.7508/jns.2016.02.005

15. Kulatunga DCM, Dananjaya SHS, Godahewa GI, Lee J. and De Zoysa M. **2017**. Chitosan Silver Nanocomposite (CAgNC) as an Antifungal Agent against *Candida albicans*, *Med.Mycol*, **55**: 213–222, DOI: 10.1093/mmy/myw053

16. Kaur P., Thakur R. and Choudhary A.**2012**.An In Vitro Study of the Antifungal Activity of Silver/Chitosan Nanoformulations against Important Seed Borne Pathogens. *Int. J. of Sci& Tec Res*, **1**(6): 83-86

17. Dubey S., Sinha DK, Murugan MS, Singh PL, Siddiqui MZ, Prasad N., Prasanna VA, Bhardwaj M, Singh BR.**2015**.Antimicrobial Activity of Ethanolic and Aqueous Extracts of Common Edible Gums against Pathogenic Bacteria of Animal and Human Health Significance, Research & Reviews: *J. of Pharm. and Nanotech.* **3**(3): 30-36.

18. Singh BR, DubeyS, and Siddiqui MZ.**2015**.Antimicrobial Activity of Natural Edible Gums, *World J Pharm Sci.* **3**(11): 2217-2221.

19. Bnuyan IA, Hindi NKK, Jebur MH, and Mahdi MA.**2015**. In vitro Antimicrobial Activity of Gum Arabic (Al Manna and Tayebat) Prebiotics against Infectious Pathogens, *Ijppr.Human,* **3**(3): 77-85.

20. Banjar MM, Khafaji AM. and Maher YA. **2017**. Antimicrobial Activity of Hydrogen Peroxide, Sesame and Gum Arabic against *Streptococcus Mutans*. *Int. J. of Health Sci. &Res*. **7**(1): 97-104.

21. Lanzilotto A, Kyropoulou M., Constable EC, Housecroft CE, Meier WP., andPalivan CG.**2018**.Porphyrin-polymer Nanocompartments: Singlet Oxygen Generation and Antimicrobial Activity, *JBIC J. of Biol Inorganic Chem*,**23**:109–122, https://doi.org/10.1007/s00775-017-1514-8.

22. DědkováK ,PeikertováP, MatějováK, Lang J., and KukutschováJ.**2012**.Study of the Antibacterial Activity of Composites Kaolinite/TiO_2, *Nanocon.*,23-25.

23. Mariselvi P. andAlagumuthu G.2015. Structural, Morphological and Antibacterial Activity of Kaolinite/TiO_2Nanocomposites, *J. Nanosci. Tech*. **1**(1): 16-18.

24. Holešováa S, Hundákováa M, and Pazdziora E.**2016**. Antibacterial kaolinite based nanocomposites, *Procedia Materials Science*. **12**:124-129.

25. Shehu Z., Danbature WL, Maisanda AS, and Musa MS.**2018**.Synthesis, Characterization and Antibacterial Activity of Kaolin/Gum Arabic Nanocomposite on Escherichia Coli and Pseudomonas Aeruginosa. *Res J. of Nanosci and Eng.* **2**(2): 23-29.