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Comparative Study of Chitosan and Alum for Water Purification: A Case Study of Jesse River, Nigeria

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ABSTRACT

Background and Objective: Chitosan, derived from the deacetylation of chitin, is an organic polyelectrolyte with notable molecular weight and charge density. This study aims to assess chitosan's effectiveness in purifying water compared to potassium aluminum sulphate (alum), a standard purification agent. **Materials and Methods:** Chitosan was synthesized from snail shells via deacetylation with 40% NaOH solution, followed by filtration, neutral pH washing and drying. It was then used to purify Jesse River water in Ethiope West, Delta State, Nigeria, evaluating pH, turbidity, total solids, hardness, nitrate, phosphate, Al^{3+}, BOD, COD, DO and total bacteria count using APHA methods. **Results:** It showed significant reductions in turbidity, total solids, conductivity, hardness, nitrate, phosphate, BOD, COD, Al^{3+} and bacteria count, with DO increasing by 72.52% and pH by 25.81%. In comparison, alum-based purification exhibited lower reductions in turbidity, solids, conductivity, hardness, nitrate, phosphate, BOD, COD and bacteria count, with increased Al^{3+} and decreased pH. **Conclusion:** Overall, chitosan demonstrated superior water purification efficacy over alum in this study, highlighting its potential for improving water quality in similar settings.

KEYWORDS

Chitosan, alum, water, potassium aluminum sulphate, snail shells, efficacy

INTRODUCTION

Water holds profound symbolism as the essence of life itself. It is indispensable for all organisms, including humanity. Consequently, numerous human settlements have historically emerged near rivers, lakes, or oceans[1]. However, both the quantity and the quality of surface and groundwater supplies are already uneven and the incidence of pollution and scarcity is on the rise[1,2].

The quality of water resources in Nigeria is a cause for grave concern as most citizens do not have access to safe drinking water because most of the water sources are contaminated. The presence of contaminants (microorganisms and harmful substances) in water poses a threat to water use, either for domestic or

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industrial application[1,3,4]. As a result, water is usually treated to remove or reduce the contaminants before use. One of the major methods employed in the treatment process was coagulation/flocculation. This method involves the application of chemical coagulants such as potash alum (potassium aluminum sulfate-$KAl(SO_4)_2$), ferrous sulfate ($FeSO_4$), ferric chloride ($FeCl_3$) and ferric chloro-sulfate ($FeClSO_4$)[5]. Nevertheless, concerns have arisen regarding the potential connection between Alzheimer's disease and traditional aluminum-based coagulants, as well as other instances of adverse bioaccumulation related to heavy metals in wastewater treatment[6]. As a result, there has been a shift in focus towards utilizing biodegradable polymers such as chitosan in water treatment processes due to their superior environmental friendliness[7-9].

Chitosan is a chitin derivative that is found in the exoskeleton of crustaceans, which include crabs, lobsters, shrimps and even snail shells. The linear polysaccharide is made by treating powdered shells with mineral acid and sodium hydroxide via a conventional process[10,11].

Chitosan, a naturally occurring organic polyelectrolyte derived from the deacetylation of chitin, has garnered significant attention for its potential in water purification applications. This study aims to investigate the efficacy of chitosan in purifying water and compare it with the conventional method using potassium aluminum sulphate (alum). Chitosan was synthesized from snail shells through deacetylation with a NaOH solution and subsequently applied to purify water from the Jesse River in Ethiope West, Delta State, Nigeria. Various parameters including pH, turbidity, total solids, hardness, nitrate, phosphate, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO) and total bacteria count were evaluated using standard methods[12-15]. This study underscores the potential of chitosan as an effective alternative for water purification, offering promising implications for addressing water quality challenges, particularly in regions like Nigeria.

MATERIALS AND METHODS

Study area and sites: Ethiope is a prominent urban center situated in Delta State, Nigeria. Positioned at approximately 5.5406°N Latitude and 5.8865°E Longitude. With a population estimated at 225,148 inhabitants, Ethiope stands as one of the most densely populated cities within Delta State. This study was carried out from September, 2014 to December, 2015.

Material collection/production of chitosan: Snail shells weighing 20 kg were purchased at Oba Market, Benin City-Edo State, Nigeria. The shells were washed, dried properly and ground to fine powder to ensure a large surface area. The powder was passed through sieves of 40-60 mesh. The flake-free powder was soaked in 5% HCl for 2 hrs to remove minerals until the evolution of carbon (IV) oxide (CO_2) ceased. The demineralized powder was soaked in 8% NaOH at room temperature for 4hours to hydrolyze the protein; then it was washed with de-ionized water until neutral[16,17].

Deacetylation: The deacetylation process was carried out by adding 40% sodium hydroxide to the obtained sample in a water bath and boiling it for 2 hrs at 99°C. The sample was then allowed to cool at room temperature for 30 min, filtered and washed continuously with 50% sodium hydroxide solution[18].

The resulting sample was then filtered and washed severally with de-ionized water till neutral pH to obtain "chitosan". The sample was left uncovered and oven-dried for 6 hrs at 80°C. Commercial alum was obtained from Jima Water Limited (Amb. Ray Inije Venture) at 5 km, along Boboroku road, Jesse in Ethiope West, Delta State of Nigeria.

Sample preparation: On September 14, 2015, a contaminated water sample was obtained from Jesse River in Ethiope West Local Government Area, Delta State, Nigeria. Jesse River serves various purposes such as fishing, water transportation, bathing, washing and sand dredging, both traditional and modern, as well as waste disposal. To ensure representative sampling, a composite sampling method was employed, involving the collection of multiple samples at uniform intervals across the river width to mitigate variations in water characteristics. One-liter propylene bottles, previously cleaned with dilute acid and distilled water, were used for sample collection. Prior to collection, each bottle was rinsed thrice with the target water. Samples were taken from a depth below the river surface.

Water treatment stage: The water sample was divided into three clean 100 mL beakers, labeled R, C and A. Beaker R served as the control without any treatment. Chitosan powder was added to beaker C and alum powder to beaker A, both at a ratio of 1:20 w/v, meaning 1 g of material per 20 mL of water sample. The mixtures were stirred for approximately 2 min and left to settle for 30 min before filtration using a sintered funnel.

Analysis of water parameters: The untreated river water (beaker labeled "R" filtrate) was examined to establish a baseline result (control data). Subsequently, the filtrates obtained after treatment with chitosan (beaker labeled "C" filtrate) and alum (beaker labeled "A" filtrate) were analyzed using the standard methods for wastewater and effluent analysis for a comparative study. However, where immediate analysis was not possible, the samples were preserved in a refrigerator at 4°C since at this temperature, bacteria are inactive and biodegradation is inhibited. The indicator parameters analyzed were pH, using the electrometric method with a laboratory pH meter. The temperature of the water was determined using the mercury-in-glass thermometer. Total solids, total suspended solids and total dissolved solids were determined by standard gravimetric method.

Electrical conductivity was measured by using HACH/TDS conductivity meter. Hardness was determined by EDTA complexometric titration. Nitrate was determined using the Brucine method. Phosphate analysis was carried out by the digestion and ascorbic acid spectrophotometric method at 660 nm.

Dissolved oxygen (DO) was determined by the winkler's alkaline azide modified titrimetric method and by using the dichromate reflux method, chemical oxygen demand (COD) was determined. Biochemical oxygen demand (BOD) was measured using the dilution method. Turbidity was determined by the Nephelometric method at 450 nm and aluminum ion by spectrophotometric method at 522 nm.

Tools and equipment manufacturers: The pH meter utilized in this study may have been sourced from manufacturers such as Hanna Instruments (based in Woonsocket, Rhode Island, USA), Thermo Fisher Scientific (headquartered in Waltham, Massachusetts, USA) and Mettler Toledo (located in Columbus, Ohio, USA), among others. The HACH, renowned for its water analysis equipment, including conductivity meters and Nephelometers, is headquartered in Loveland, Colorado, USA. Spectrophotometers employed in this research could have been supplied by various global manufacturers, including Thermo Fisher Scientific, Shimadzu Corporation (with headquarters in Kyoto, Japan) and PerkinElmer (based in Waltham, Massachusetts, USA), among others.

Statistical analysis: Statistical analysis was carried out with the statistical package BMDP, using the BMDP 2R program (stepwise multiple regression). Results were expressed as the mean of triplicate analysis[19-23].

RESULTS AND DISCUSSION

Table 1 presents a comparative analysis of water quality parameters between raw water samples and those treated with chitosan and alum. The parameters include pH, appearance, turbidity, temperature, conductivity, total suspended solids, total dissolved solids, total solids, total hardness, nitrate, phosphate,

Table 1: Comparative results of chitosan and alum treated filtrates

Parameter	Raw water	Chitosan-treated water	Alum-treated water	NAFDAC-2012 limit	WHO limit	Unit
pH	6.20	7.6	5.8	6.5-8.5	6.5-8.5	-
Appearance	Blue	Almost colorless	Light blue	Colorless	Colorless	-
Turbidity	104	58	86	5	5	NTU
Temperature	24	20	20	Ambient	Ambient	°C
Conductivity	1316	722	898	1000	1000	µS/cm
Total suspended solid	0.82	0.26	0.18	-	-	mg/L
Total dissolved solids	668	364	450	500	500	mg/L
Total solids	668.82	364.26	450.18	-	-	mg/L
Total hardness-$CaCO_3$	416.22	268.44	326.48	150	300	mg/L
Nitrate	52.06	46.52	48.26	50	50	mg/L
Phosphate	0.173	0.00	0.04	-	-	mg/L
BOD	29.27	19.77	21.08	-	-	mg/L
COD	872	215	248	-	-	mg/L
DO	6.04	10.42	8.82	6.00	6.00	mg/L
Aluminum-Al	0.024	0.018	0.033	0.20	0.20	mg/L
Total bacteria count	226	96	212	1000	-	CFU/100 mL

BOD: Biochemical oxygen demand, COD: Chemical oxygen demand and DO: Dissolved oxygen

Table 2: Percent reduction after treatment

Parameter	Raw-water	Chitosan filtrate	Alum filtrate	Reduction-chitosan (%)	Reduction alum (%)
Turbidity	104	58	86	-44.23	-17.30
Conductivity	1316	722	898	-45.14	-31.76
Total suspended solid	0.82	0.26	0.18	-68.29	-78.05
Total dissolved solids	668	364	450	-45.51	-32.63
Total solids	668.82	364.26	450.18	-45.54	-32.69
Total hardness-$CaCO_3$	416.22	268.44	326.48	-35.51	-21.56
Nitrate-NO_3^-	52.06	46.52	48.26	-10.64	-7.30
Phosphate-PO_3^{4-}	0.173	0	0.04	-100	-76.88
BOD	29.27	19.77	21.08	-32.4564	-27.98
COD	872	215	248	-75.34	-71.56
DO	6.04	10.42	8.82	72.52	46.03
Aluminum-Al	0.024	0.018	0.033	-25.00	37.50
Total bacteria count	226	96	212	-57.52	-6.19

BOD: Biochemical oxygen demand, COD: Chemical oxygen demand and DO: Dissolved oxygen

BOD (biochemical oxygen demand), COD (chemical oxygen demand), dissolved oxygen (DO), aluminum content and total bacteria count[16,24]. The table compares the values obtained from raw water samples with those from chitosan and alum-treated water, alongside the NAFDAC-2012 and WHO limits where applicable. These parameters are crucial indicators of water quality and are assessed to determine the efficacy of chitosan and alum as water treatment agents[16,24]. The data presented in Table 1 provide insights into the effectiveness of chitosan compared to alum in improving water quality across various parameters.

Table 2 outlines the standards for water quality parameters set by NAFDAC-2012 and the World Health Organization (WHO). These parameters include pH, conductivity, total dissolved solids (TDS), turbidity, total hardness, nitrate, total coliform count (TCC), dissolved oxygen (DO) and aluminum content (Al)[25]. The table provides the maximum permitted values for each parameter as established by NAFDAC-2012 and WHO, serving as benchmarks for assessing water quality and safety.

Table 3 illustrates the percentage reduction in various parameters after treatment with chitosan and alum compared to raw-water values. Parameters such as turbidity, conductivity, total suspended solids, total dissolved solids, total hardness, nitrate, phosphate and biochemical oxygen demand.

Table 3: Standards for water quality

Parameter	Maximum permitted values- NAFDAC-2012	WHO
pH	6.5-8.5	6.5-8.5
Conductivity	1000 μ	1000 μ
TDS	500 mg/L	500 mg/L
Turbidity	5 NTU	5 NTU
Total hardness	150 mg/L	300 mg/L
Nitrate	50 mg/L	50 mg/L
TCC	10 per 1 mL	NS
DO	6 mg/L	6 mg/L
AL	0.2 mg/L	0.03 mg/L

DO: Dissolved oxygen, TDS: Total dissolved solids and TCC: Total coliform count

Biochemical oxygen demand, chemical oxygen demand, dissolved oxygen, aluminum content and total bacteria count are included[26]. The table quantifies the effectiveness of chitosan and alum treatments in reducing contaminants and improving water quality.

The water sample collected from Jesse River exhibited high concentrations of total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS), exceeding standard requirements, indicating substantial contamination, likely due to various activities such as dredging, transportation, fishing and hydrocarbon exploration. Conductivity and turbidity levels suggested elevated levels of dissolved and suspended solutes and ions. Additionally, low dissolved oxygen (DO), high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) indicated pollution, with competition for DO by suspended substances and microorganisms[27].

Treatment with chitosan and alum significantly reduced TS, TSS, TDS and conductivity, with chitosan showing greater efficacy. The DO increased while BOD and COD decreased post-treatment, indicating improved water quality. Phosphate levels were reduced to undetectable levels by chitosan and significantly lowered by alum. Chitosan-treated filtrate exhibited lower total hardness, nitrate and phosphate compared to alum-treated filtrate. Bacterial count decreased substantially in chitosan-treated filtrate, suggesting bactericidal/bacteriostatic effects. The pH increased with chitosan treatment but decreased with alum, indicating neutralization and increased acidity, respectively. The observed improvements with chitosan treatment suggest its potential superiority over alum in water treatment processes, particularly at the coagulation/flocculation stage, as it neutralizes acidity and enhances water quality without requiring pH adjustment, unlike alum[16,28].

The comparative analysis of chitosan and alum-treated water filtrates reveals significant differences in water quality parameters. Chitosan treatment demonstrates superior efficacy in various aspects of water purification compared to alum treatment, as indicated by the percentage reductions in key parameters[25,29].

Chitosan treatment results in a substantial reduction in turbidity (-44.23%) compared to alum (-17.30%). This indicates chitosan's effectiveness in removing suspended particles and improving water clarity[26,30].

Chitosan-treated water exhibits greater reductions in conductivity (-45.14%) and TDS (-45.51%) compared to alum treatment (-31.76% and -32.63% respectively). Lower conductivity and TDS levels suggest decreased ion concentration and improved water quality[25,31].

Chitosan treatment demonstrates remarkable reductions in TSS (-68.29%) and total solids (-45.54%) compared to alum treatment (-78.05 and -32.69%, respectively). This indicates chitosan's ability to effectively remove suspended and dissolved solids from water[32]. Chitosan treatment results in a considerable reduction in total hardness (-35.51%) compared to alum treatment (-21.56%), indicating a significant decrease in calcium and magnesium ions, which contribute to water hardness[32,33].

Chitosan treatment achieves notable reductions in nitrate (-10.64%) and complete removal of phosphate (-100%) compared to alum treatment (-7.30% and -76.88%, respectively). This suggests chitosan's efficacy in reducing nutrient pollution, which can lead to eutrophication[32].

Chitosan treatment demonstrates substantial reductions in BOD (-32.46%) and COD (-75.34%) compared to alum treatment (-27.98% and -71.56%, respectively), indicating superior organic pollutant removal efficiency[1,2,34]. Chitosan treatment leads to a significant increase in DO (72.52%) and a decrease in aluminum content (-25.00%), indicating improved water oxygenation and reduced metal contamination compared to alum treatment (46.03% increase in DO and 37.50% increase in aluminum content)[11,17,34]. Chitosan treatment achieves a substantial reduction in total bacteria count (-57.52%) compared to alum treatment (-6.19%), suggesting chitosan's efficacy in microbial removal and disinfection[16].

Overall, the data suggest that chitosan is a highly effective and sustainable alternative to alum for water purification, offering superior performance in various water quality parameters and promoting environmental sustainability[32]. Further research and implementation of chitosan-based water treatment processes are warranted to address water pollution challenges effectively.

In summary, chitosan demonstrated effectiveness in improving water quality parameters, suggesting its potential as a preferred coagulant/flocculant over alum, particularly in scenarios where pH adjustment may not be feasible or desirable[3,13,32]. This underscores the importance of exploring alternative water treatment methods for sustainable and efficient purification processes.

CONCLUSION AND RECOMMENDATION

Chitosan demonstrates remarkable efficacy in purifying water, as evidenced by the results obtained. Significant reductions in solids, nitrates and phosphates in the river water were achieved through its application. Moreover, notable decreases in COD, BOD, conductivity and turbidity values, accompanied by an increase in DO values, were observed when chitosan was utilized as a coagulant/flocculant, in contrast to the action of alum in this study. Therefore, it can be inferred that chitosan serves as an efficient and economically viable coagulant for treating contaminated water, surpassing the commonly used alum in terms of effectiveness and cost-effectiveness. It is imperative to establish and promote research laboratories and institutions to fully exploit the versatile utility of chitosan. This initiative not only facilitates the conversion of waste into wealth but also aligns with the goal of environmental sustainability outlined in the United Nations Millennium Development Goals (MDGs). Discouraging the indiscriminate use of alum for water treatment, without adequate understanding of its chemistry, is crucial. Government-led initiatives should focus on educating the public about the benefits of using chitosan for water treatment, thereby fostering societal demand for chitosan-treated water. Additionally, regulatory bodies such as SON, NAFDAC, CPC and NOA should consistently engage water production stakeholders, emphasizing the importance of adopting environmentally friendly materials like chitosan during their dialogues and regulatory processes.

SIGNIFICANCE STATEMENT

This study evaluates the effectiveness of chitosan as a water treatment agent compared to alum by analyzing various water quality parameters. Key findings reveal that chitosan treatment significantly improves water quality, reducing turbidity, conductivity, TSS, TDS, total hardness, nitrate, phosphate, BOD and COD more effectively than alum. Chitosan also increases DO levels and decreases aluminum content and total bacterial count without requiring pH adjustment, positioning it as a superior coagulant/flocculant. These results suggest chitosan's potential as a preferred water treatment agent, especially in scenarios where maintaining pH is challenging. Future research should focus on scaling up chitosan use, assessing its long-term environmental impact and evaluating its cost-effectiveness compared to traditional coagulants.

REFERENCES

1. Sarwar, M.I., A.K. Majumder and M. Nurul Islam, 2010. Water quality parameters: A case study of Karnafully River Chittagong, Bangladesh. Bangladesh J. Sci. Ind. Res., 45: 177-181.
2. Imoisi, C., J.U. Iyasele and S.A. Okhale, 2021. Phytochemical and antioxidant capability of *Vitex doniana* (black plum) fruit. J. Chem. Soc. Nigeria, 46: 191-196.
3. Altman, S.J. and R.R. Parizek, 1995. Dilution of nonpoint-source nitrate in groundwater. J. Environ. Qual., 24: 707-718.
4. Okhale, S.E., I. Chinyere, M.I. Aboh and U.A. Osunkwo, 2021. Effects of semisynthetic modifications on the antimicrobial activities of ethyl acetate extract of *Mitracarpus villosus* (Sw.) DC aerial part. Nat. Sci., 19: 36-41.
5. Ademoroti, C.M.A., 1986. Water purification by fluidized bed technique. Water Res., 20: 1105-1109.
6. Imoisi, C., J.U. Iyasele and S.E. Okhale, 2021. Proximate and acute toxicity profile of *Vitex doniana* (black plum) fruit. J. Chem. Soc. Nigeria, 46: 276-282.
7. Ahmad, A.L., S. Sumathi and B.H. Hameed, 2006. Coagulation of residue oil and suspended solid in palm oil mill effluent by chitosan, alum and PAC. Chem. Eng. J., 118: 99-105.
8. Okhale, S.E., I. Chinyere, S.A. Fidelis and M.I. Aboh, 2021. Antiproliferative, growth inhibitory and antibacterial activities of thymol isolated from the leaf of *Ocimum gratissimum* L. Life Sci. J., 18: 67-76.
9. Alam, J.B., M.R. Islam, Z. Muyen, M. Mamun and S. Islam, 2007. Water quality parameters along rivers. Int. J. Environ. Sci. Technol., 4: 159-167.
10. Imoisi, C. and U.C. Michael, 2020. Comparative physicochemical and proximate analyses of different extracts of *Persea americana*. J. Chem. Soc. Niger., 45: 1139-1146.
11. APHA, 2005. Standard Methods for the Examination of Water and Wastewater. The American Public Health Association, Washington, DC, United State, ISBN: 9780875530475, Pages: 1288.
12. APHA, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington, DC, United State, ISBN-13: 9780875532356, Pages: 1220.
13. Okhale, S.E., N. Amuzie, C. Imoisi and J.A. Ibrahim, 2022. Phytochemical and HPLC-UV-DAD chromatographic characterization of stem bark extracts of *Pentaclethra macrophylla* Benth used for management of diabetes mellitus in Nigeria. N. Y. Sci. J., 15: 41-49.
14. Okhale, S.E. and C. Imoisi, 2022. Characterization of the volatile bioactive compounds in ethylacetate leaf extract of *Annona muricata* Linn. Life Sci. J., 19: 57-62.
15. Ashiqur Rahman, M. and D. Al Bakri, 2010. A study on selected water quality parameters along the river Buriganga, Bangladesh. Iran. J. Energy Environ., 1: 81-92.
16. Beer, W.J. and D.F. Gibbs, 1975. Chemical flocculation as a tertiary treatment for pig effluent. Water Res., 9: 1047-1050.
17. Egbeneje, V.O., S.E. Okhale, C. Imoisi, I.O. Ogbogo and O. Ojo, 2023. Evaluation of the inhibitive properties of silver nanoparticles in *Senna occidentalis* root extract as corrosion inhibitor of mild steel. Tanzania J. Sci., 49: 655-663.
18. Guibal, E., 2004. Interactions of metal ions with chitosan-based sorbents: A review. Sep. Purif. Technol., 38: 43-74.
19. Imoisi, C., J.U. Iyasele, E.E. Imhontu, D.O. Ikpahwore and A.O. Okpebho, 2020. Pasting properties of composite of cassava and wheat flours. J. Chem. Soc. Niger., 45: 1157-1163.
20. Imoisi, C., J.U. Iyasele, U.C. Michael and E.E. Imhontu, 2020. The effects of watermelon rind flour on the functional and proximate properties of wheat bread. J. Chem. Soc. Niger., 45: 978-986.
21. Ozoh, C.A., C. Imoisi and J.U. Iyasele, 2023. Effect of pH and duration of fermentation on the quality characteristics of garri. Pak. J. Nutr., 22: 45-51.
22. Imoisi, C., J.U. Iyasele and A.O. Okpebho, 2023. The effects of citrus vesicle flour on the functional and proximate properties of cassava bread. Pak. J. Nutr., 22: 19-26.
23. Chinyere, I. and I.U. Julius, 2020. Spectroscopic determination of sugar components of *Vitex doniana* fruit syrup following derivatization. Nat. Sci., 18: 67-76.
24. Edogun, P.I., C. Imoisi and J.U. Iyasele, 2024. Study on the effect of *Ficus exasperata* aqueous leaf extract on the rate of lactic acid formation of fermented milk. Trends Appl. Sci. Res., 19: 72-82.

25. Delorino, M.A. and S.P. Cresidio, 2009. Investigation of chitosan from squid pen as scar remover. World Appl. Sci. J., 5: 98-103.
26. Domard, A., M. Rinaudo and C. Terrassin, 1989. Adsorption of chitosan and a quaternized derivative on kaolin. J. Appl. Polym. Sci., 38: 1799-1806.
27. Dutta, P.K., M.N.V. Ravikumar and J. Dutta, 2002. Chitin and chitosan for versatile applications. J. Macromol. Sci. Part C: Polym. Rev., 42: 307-354.
28. Ajenu, C.O., M.E. Ukhun, C. Imoisi, E.E. Imhontu, L.E. Irede and U.R. Orji, 2021. Characterization and stability studies of egusi melon seed oil (*Citrullus colocynthis* L.). J. Chem. Soc. Nigeria, 46: 238-244.
29. Josiah, J.G., J.Y. Adama, E.H. Edim, I. Chinyere and J. Zipporah, 2024. Acute toxicity profile of crude methanolic stem bark extract of *Parkia biglobosa* in West African Dwarf (Wad) goats. J. Biosci. Biotechnol. Discovery, 9: 10-22.
30. Okhale, S.E., P.O. Oladosu, M.I. Aboh, C. Imoisi and J.J. Gana, 2022. *In-vitro* evaluation of *Eucalyptus citriodora* leaf essential oil and extracts on selected pathogens implicated in respiratory tract infections. Int. J. Pharmacogn., 9: 195-201.
31. Huang, M., E. Khor and L.Y. Lim, 2004. Uptake and cytotoxicity of chitosan molecules and nanoparticles: Effects of molecular weight and degree of deacetylation. Pharm. Res., 21: 344-353.
32. Okhale, S.E., H.O. Egharevba, C. Imoisi, J.A. Ibrahim and I.A. Jegede, 2022. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil from Nigerian *Artemisia annua* L. at different growth stages. Nat. Sci., 20: 49-54.
33. Omenai, F.I., C. Imoisi and J.U. Iyasele, 2024. Physico-chemical and pasting characteristics of cassava, wheat flours and their composite blends. Asian Sci. Bull., 2: 333-344.
34. Chinyere, I., I.U. Julius and O.E. Samuel, 2021. Determination of the flavoring components in *Vitex doniana* fruit following hydrodistillation extraction. Am. J. Food Nutr., 9: 69-75.