Green Synthesis and Antimicrobial Potency of Silver Nanoparticles from Ocimum gratissimum Leaf Extract on Clinical Isolates of Escherichia coli and Klebsiella Pneumoniae Isolated from Patients in a Tertiary Health Hospital, Abakaliki, Ebonyi State, Nigeria.

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Abstract

Background: Nanoparticles are gaining importance in research especially in the field of medicine. This study aimed at synthesizing silver nanoparticles from Ocimum gratissimum and determining their therapeutic potentials against clinical isolates of Escherichia coli and Klebsiella pneumoniae

Methods: Silver nanoparticles were synthesized by mixing aqueous leaf extract of Ocimum gratissimum and 1 mM of silver nitrate at the ratio of 1:4, heated on a sand bath at 60°C for 30 minutes and observed for colour change. The formation of silver nanoparticles was confirmed by observing the optical properties of the solution using ultraviolet visible spectroscope. Antibacterial potency of the synthesized silver nanoparticles against clinical isolates of Escherichia coli and Klebsiella pneumoniae was assessed using agar well diffusion techniques.

Results: Aqueous leaf extract of Ocimum gratissimum was able to synthesize silver nanoparticles as colour changed from dark orange to dark brown. The synthesized silver nanoparticles of Ocimum gratissimum leaf extract showed higher activity against the isolates compared to ethanoic leaf extract alone. The result of two different concentrations of silver nanoparticles against Escherichia coli showed that at 100 µg/ml and 150 µg/ml an inhibition zone diameter of 20 mm and 21 mm was observed while in Klebsiella pneumoniae isolates, an inhibition zone diameter of 18 mm and 20 mm was observed at 100 µg/ml and 150 µg/ml concentrations respectively.

Conclusion: This study established the antibacterial potency of Ocimum gratissimum synthesized silver nanoparticles against clinical isolates of Escherichia coli and Klebsiella pneumoniae and should be processed further for use against these infections.

Keywords: Ocimum gratissimum, Silver nanoparticle, Antibacterial activity, Escherichia coli, Klebsiella pneumoniae

Introduction

The study of nanoparticles (NPs), its synthesis and applications have over the years gained more relevance globally owing to their characteristics, especially their small size which measures between 1 to 100 nm, relatively large area surface and reactivity. These characteristics enhance their therapeutic use in different dosing routes and dosages. Nanoparticles can be produced from several sources such as solid phase, liquid phase or gaseous phase. Among the NPs, Silver Nanoparticles (AgNPs) have gained the widest attention in recent times as a result of their unique properties including physical, optical and electrical properties as well as their thermal behavour.[1-4] Another important property of AgNPs is their ability to be synthesized using several synthetic methodologies such as biological, chemical and physical methods.[5] However, their application is mostly found in biological products, catalysts and chemical sensors.[6,7,8,9] Of special interest in the application of AgNPs are the effective usage in fabrics, biomedical devices and wound treatment due to their bacteria-repelling-properties and antimicrobial coating.[10,11,12,13] These unique properties are largely influenced by the size, shapes and methods of synthesis.[14] In the investigation of antibacterial potency of AgNPs, scientists have engaged in the study of toxicity properties of AgNPs on bacterial isolates, colony forming units (CFU) and most importantly inhibition zone diameter (IZD).[15,16] The large area surfaces of NPs enhances their ability to interact well with bacterial cells when compared to other larger particles.[14] It has been reported that AgNPs of 5 nm size have higher antibacterial properties against E. coli, S. aureus, when compared to larger particles of 7 to 10 nm size at similar bacterial concentration.[17] The shape of AgNPs has also been shown to be an important factor in considering its antibacterial properties.[18]

The synthesis of AgNPs using several plant extracts is currently being studied by researchers as a result of their availability in abundance, simplicity, antibacterial potency and the high level of antibiotic resistance phenotype expressed by Gram negative organisms.[19,20,21] Several plant parts, such as roots, stems, leaves, fruits and seeds have been suggested for use in the green synthesis of AgNPs. Among them, the exploration of leaf extract as a stabilizing and reducing agent in synthesizing nanoparticles especially AgNPs is preferred as a result of their availability, eco-friendly procedure, minimum use of energy and large consumption rate. The choice of Ocimum gratissimum popularly known as scent leaf or basil in the green synthesis of AgNPs was due to the wide acceptance and usage as condiment and as a sedative in Nigeria.[22] It is also widely used as traditional medicine for the treatment of various infections, headache, abdominal pain, cough, cold, and bronchitis.[23,24] The increasing reports of antibiotic resistant strains and non-production of newer antibiotics to combat the ever increasing resistant strains of microorganisms have led scientists to look for alternative therapies that are eco-friendly and-readily available with a high therapeutic potential. The aim of this study was therefore to synthesize silver nanoparticle from Ocimum gratissimum and ascertain their therapeutic potential against clinical isolates of Escherichia coli and Klesiella pneumoniae obtainted from in-patients at Alex Ekwueme-Federal University Teaching Hospital, Abakaliki, Ebonyi State.

Methodology

Study area and study design

This study was carried out at Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA), Ebonyi State, Nigeria. AE-FUTHA is a tertiary care hospital in Abakaliki, Ebonyi State and serves as a referral hospital to all other private, mission hospitals and general hospitals in Ebonyi State. A total of 10 clinical isolates (made up of 5 isolates of Escherichia coli and 5 isolates of Klesiella pneumoniae were collected from microbiology laboratory unit of Alex Ekwueme-Federal University Teaching Hospital Abakaliki (AE-FUTHA), Ebonyi State and was taken in an agar slant to the laboratory unit of Applied microbiology Department of Ebonyi State University Abakaliki for re-confirmation and laboratory analysis. This study was carried out over a period of two months between June and July, 2023.

Media used: This includes: nutrient agar, nutrient broth, MacConkey Agar (Merck, German) Mueller- Hinton agar (Lancashire, UK),

Equipments used: The microscope (Olympus Optical CO. Ltd, U.K.), Autoclaves (Search Tech, England), Incubator, UV spectrophotometer, centrifuge (Lincoln Mark, England), Refrigerator (Haier Thermoccol), Weighing balance (Atrontec Electronic Tech Co., Ltd).

Reagents used: Gram staining reagents used include; Crystal violet, acetone – alcohol decolorizer, lugol's iodine, neutral red (Hardy Diag. Gram stain kits and Reagents). Other reagents used include; distilled water, normal saline, immersion oil, EDTA salt, oxidase reagent.

Collection of fresh leaves of Ocimum gratissimum (OG)

Fresh leaves of O. gratissimum (scent leaf) was bought from market women at Margareth Umahi international market, Abakaliki, Ebonyi State.

Nanoparticles used: AgNPs was used for this study by mixing O. gratissimum leaf extract with silver nitrate solution, followed by monitoring for colour change.

Preparation of O. gratissimum leaf extract and 1mM AgNO3

The fresh leaves of O. gratissimum was washed thoroughly (up to 3 times) with tap water and finally with distilled water to remove any visible dust particles. The leaves were then air-dried at room temperature for 72 hours to remove moisture contents before it was grinded into fine particles using electrical blender. The fine particles of O. gratissimum leaf extract was then taken to Laboratory unit of Applied Microbiology Department, Ebonyi State University, Abakaliki for analysis. Subsequently, a total of 10 grams of O. gratissimum was transferred to sterile 500 mL conical flask. Distilled water 200 mL was added to the flask and heated at 60 ^{o}C for about 10 min and incubated on sand bath for 30 min to facilitate the formation of aqueous extract. The extract was then filtered using Whatman No 1 filter paper and the filtrate stored at 4 ^{o}C for further use. Silver nitrate ($AgNO_3$, Sigma Aldrich, USA), 0.0421 gm was added to 100 mL of double distilled water and dissolved thoroughly. The solution obtained was then transferred to an amber coloured bottle to prevent autoxidation of silver.[25]

Determination and synthesis of AgNPs

The aqueous leaf extract of O. gratissimum and 1 mM of $AgNO_3$ was mixed in the ratio of 1: 4 and heated on a sand bath at 60 ^{o}C for 30 min until change in colour was observed from orange brown to dark brown colour. The colour change indicated the formation of AgNPs by O. gratissimum leaf (OGL) extract to form OGL-AgNPs solution.[26]

Characterization of biosynthesized AgNPs

The change in colour of O. gratissimum leaf extract after addition of 1 mM of $AgNO_3$ indicated the formation of AgNPs by O. gratissimum leaf (OGL) extract to form OGL-AgNPs solution. Furthermore, the formation of AgNPs was confirmed by studying the optical properties of the solution using a UV visible spectroscope (UV 1800V of Shimadzu, Japan).[26,27]

Standardization of test inoculum

Overnight cultures of the isolates were used for determination of antimicrobial activity. Each of the isolates was standardized using colony suspension method.[28] The test organisms from nutrient agar plates, incubated at 37 ^{0}C for 24 hours were suspended in saline solution (0.85 % NaCl). The density of each isolate suspension was matched with 0.5 McFarland standards equivalent to give a resultant concentration of 1.0×10^{6} cfu/ml.[29]

Preparation of McFarland Standard

The 0.5 MacFarland turbidity standard equivalent was prepared by adding 1ml of conc tetraoxosulphate (vi) acid ($H_2SO_{4)}$ to 99 ml of distilled water. A 0.5 g of dehydrated barium chloride ($BaCl_2.2H_20$) was dissolved in 50 ml of distilled water in a separate reaction flask. Then, 0.6 ml of barium chloride solution was added to 99.4 ml of tetraoxosulphate (vi) acid solution in a separate test tube and the reaction mixture was properly mixed to form turbidity equivalent to 0.5 MacFarland turbidity standard. Small portion of the turbid solution was transferred to test tube similar to the tube used for preparing the test organism and stored at room temperature.[30]

Antibacterial Activity

The antibacterial assays of the AgNPs against E. coli and K. pneumoniae was assessed by using agar well diffusion techniques. The clinical isolates of E. coli which was obtained from AE- FUTHA was re-confirmed morphologically and through their biochemical characteristics as described by Cheesbrough.[30] A 0.5 MacFarland standard equivalent of overnight inoculums of the test isolates which was previously sub-cultured onto nutrient agar was inoculated onto the surface of Mueller -Hinton agar plates by streaking and allowed for 30 mins for pre-diffusion. A six (6) mm diameter cork borer was used to bore 5 holes on the surface of the Mueller Hinton agar plates previously streaked with the inoculums. The central well was loaded with the prepared O. gratissimum leaf (OGL) extract, followed by distilled water as a negative control, and two different concentrations of prepared silver nanoparticles (100 µg/ml and 150 µg/ml) which was inter-switched with the pure silver nitrate, loaded clockwise from top. The plates were then incubated overnight at room temperature. The inhibition zone diameter (IZD) formed around the wells was measured in mm using meter rule and results tabulated to compare the antimicrobial activity of the AgNPs with that of pure extract and silver nitrate.[26]

Results

Morphologic, microscopic and biochemical characteristics of clinical isolates of E. coli and K. pneumoniae of the 10 clinical isolates of E. coli and K. pneumoniae collected from AE-FUTHA, a total of 6 isolates was confirmed as E. coli while 4 isolates was re-confirmed as K. pneumoniae following culture, Gram stain and biochemical characteristics of the isolates (Table 1).

Antimicrobial potentials of the synthesized AgNPs against clinical isolates of E. coli. The determination of the antibacterial potential of the synthesized AgNPs against the bacterial isolate of E. coli showed that there is no observable inhibition on distilled water (DW) and silver nitrate solution ($AgN0_3$). However, the aqueous ethanoic leaf extract of O. gratissimum was able to inhibit the growth of the isolated E. coli with inhibition zone diameter (IZD) of 18 mm, while the two concentrations of the synthesized AgNPs had IZD of 20 mm and 21 mm in 100 µg/ml and 150 µg/ml respectively (Table 2).

Antimicrobial potentials of the synthesized AgNPs against clinical isolates of K. pneumoniae

The antibacterial activities of the synthesized OG-AgNPs against clinical isolates of K. pneumoniae revealed inhibition of K. pneumoniae isolates with IZD of 18 mm and 20 mm at the concentrations of 100 µg/ml and 150 µg/ml respectively. The ethanol aqueous leaf extract of O. gratissimum had IZD of 16 mm. No inhibition was observed in distilled water and $AgN0_3$ (Table 3).

Table 1: Morphologic, microscopic and biochemical c haracteristics of clinical isolates of *E. coli* and *K. pneumoniae*

Bacterial isolate (n=10)	Morphology	GS	CAT	CT	OT	IT	MT	Number and (%) Confirmed isolate
EC 1-5	Smooth shinning pink colonies on MacConkey agar. Lactose fermenter	-	+	-	-	+	+	6(60.0)
KP1-5	Pink, mucoid colonies with foul smelling odour on MacConkey agar. Non -lactose fermenter.	-	+	+	-	-	-	4(40.0)

Key: GS= Gram stain, CAT= Catalase test, CT= Citrate Test, OT= Oxidase Test, IT= Indole Test, MT= Methyl red test.

Table 2: Antimicrobial potential of the synthesized AgNPs against clinical isolates of *E. coli*

Bacterial isolate	Inhibition Zone Diameter (mm)				
E. coli	**OGEE**	**DW**	**OG-AgNPs (100 µg/ml)**	**$AgN0_3$**	**OG-AgNPs (150 µg/ml)**
	18	NI	20	NI	21

Key: OGEE= *Ocimum gratissimum* ethanol extract; DW= Distilled water; OG -AgNPs= *Ocimum gratissimum*-silver nanoparticles**,** NI**=** No Inhibition

Table 3 : Antimicrobial potentials of the synthesized AgNPs against clinical isolates of *K. pneumoniae*

Bacterial Isolate	Inhibition Zone Diameter (mm)				
K. pneumoniae	**OGEE**	**DW**	**OG-AgNPs (100 µg/ml)**	**$AgN0_3$**	**OG-AgNPs (150 µg/ml)**
	16	NI	18	NI	20

Key: OGEE= *Ocimum gratissimum* ethanol extract; DW= Distilled water; OG -AgNPs= *Ocimum gratissimum*-silver nanoparticles**,** NI= No Inhibition

Discussion

In Nigeria, the use of medicinal plants which have antimicrobial properties has evolved due to increased antibiotic resistance. The result of antibacterial potency of aqueous ethanoic leaf extract of *O. gratissimum* showed increased inhibition of *E. coli* with IZD of 18 mm, while the $AgNO_3$ had 16 mm inhibition against the isolated *E. coli*. However, the two concentrations of the synthesized OG-AgNPs had the IZD of 20 and 21 (mm) at 100 µg/ml and 150 µg/ml respectively against the isolated *E. coli*.

This result is in line with the results of other studies in the literature who also revealed that *O. gratissimum* synthensized AgNPs have the ability to inhibit the growth of microorganisms including *S. aureus*, *E. coli* etc**.**[31,32] It is therefore important to utilize this novel plant as a food condiment as well as exploiting the active ingredients for other health benefits especially for microbial growth control. Furthermore, the inhibition of *E. coli* by ethanoic extract of *O. gratissimum* was not unexpected as report from another study have shown that aqueous ethanoic extract of *O. gratissimum* has antibacterial properties against members of the *Enterobacteriaceae*.[33] Also, the results of other studies carried out elsewhere,[34,35] have been confirmed by the result of this study where they reported antibacterial properties of *O. gratissimum* against four clinical bacteria isolates namely: *Escherichia coli, Proteus mirabilis, Staphylococcus aureus and Pseudomonas aeruginosa*.

The result of this study further revealed that both the ethanoic leaf extract of *O. gratissimum* and OG-AgNPs have antibacterial potency against clinical isolates of *K. pneumoniae*. Also, studies have shown that both aqueous and ethanoic leaf extract of *O. gratissimum* have an antibacterial activities against pathogenic strains of microorganisms and therefore can be used in the treatment of several diseases.[36,37] Other studies, have also shown that medicinal plants and their bioactive compounds are useful in the treatment or prevention of life-threatening and chronic diseases including strokes, arthritis, diabetes and cancer.[38,39,40] These studies further support the result of this finding where the ethanoic leaf extract of *O. gratissimum* and the *O. gratissimum* synthesized AgNPs are able to inhibit the growth of *K. pneumoniae* isolates tested with IZD of 16 mm for the leaf extract of *O. gratissimum,* 18 mm and 20 mm for the two different concentration of OG-AgNPs tested. It is therefore imperative to state that OG-AgNPs can further be processed and used for treatment of bacterial infections especially infections due to *E. coli* and *K. pneumoniae* rather than relying on the leaf extract alone which have shown a lower IZD in both isolates of *E. coli* and *K. pneumoniae* tested. The findings of this study present the opportunity of utilizing the active ingredients of *O. gratissimum* for green synthesis of AgNPs which is eco-friendly, and also can be used for the treatment of bacterial pathogens.

This study enhances the understanding of the efficacy of local plants for the synthesis of AgNPs

which have shown to be very effective in the treatment of bacterial infections. The use of local plants in the green synthesis of AgNPs has attracted interests of various researchers globally owing to their unique and appealing properties especially their ability to inhibit microbial growth and therefore should be explored in Nigeria to control the menace of antibiotic resistance. However, despite the overwhelming progress achieved in this study, it is important that the efficacy of this novel antibacterial agent be tested on other bacteria species, viral and fungal pathogens. Also, dosage and duration of administration needed established.

Conclusion

The ever increasing number of pathogenic strains of microorganism has generated the need to find new antibiotic materials and newer ways to combat these overwhelming bacterial infections. *Ocimum gratissimum* are constantly being explored by scientist and other research community as it is used as condiments, and traditionally for the treatment of various ailments. *Ocimum gratissimum* was used to synthesize AgNPs. This synthesized OG-AgNPs inhibited the growth of *Escherichia coli* and *Klebsiella Pneumoniae* isolates. Therefore, *Ocimum gratissimum* synthesized silver nanoparticles should be explored and exploited as source of new drugs against the increasing number of antibiotic resistant strains. This study therefore recommends the use of this novel agent for treatment of bacterial infections especially as the green synthesis of OG-AgNPs is eco-friendly and has been shown to have increased inhibition against the clinical isolates of *E. coli* and *K. pneumoniae* tested compared to ethanol extract of *O. gratissimum* alone.

Conflicts of Interest

Authors declare no conflicts of interest

References

1. Ashraf JM, Ansari MA, Khan HM, Alzohairy MA, Choi I. Green synthesis of silver nanoparticles and characterization of their inhibitory effects on ages formation using biophysical techniques. Sci Rep. 2016; 6:1-10.
2. Nadagouda MN, Iyanna N, Lalley J, Han C, Dionysiou DD, Varma RS. Synthesis of silver and gold nanoparticles using antioxidants from blackberry, blueberry, pomegranate, and turmeric extracts. ACS Sustain Chem Eng. 2014; 2: 1717-1723.
3. Gangopadhyay P, Kesavamoorthy R, Bera S, Magudapathy P, Nair KGM, Panigrahi BK et al. Optical absorption and photoluminescence spectroscopy of the growth of silver nanoparticles. Phys Rev Lett. 2005; 94: 1-7. Doi: 10.1103/phyRevLett.94.047403.
4. Park M, Im J, Shin M, Min Y, Park J, Cho H, et al. Highly stretchable electric circuits from a composite material of silver nanoparticles and elastomeric fibres. Nat Nanotechnol. 2012; 7: 803-809.
5. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Research in Pharmaceutical Sciences. 2014; 9(6): 385-406.
6. McFarland AD, Van Duyne RP. Single silver nanoparticles as real-time optical sensors with zeptomole sensitivity. Nano Lett. 2003; 3: 1057-1062.
7. Kumar A, Vemula PK, Ajayan PM, John G. Silvernanoparticle- embedded antimicrobial paints based on vegetable oil. Nat Mater. 2008; 7: 236-241.
8. Atwater HA, Polman A. Plasmonics for improved photovoltaic devices. Nat Mater. 2010; 9: 205-213.
9. Luis L-MJ, Borjas-Garcia SE, Esparza R, Rosas G. Synthesis and catalytic evaluation of silver nanoparticles synthesized with Aloysia triphylla leaf extract. J Clust Sci. 2016; 27: 1989-1999.
10. Lee HY, Park HK, Lee YM, Kim K, Park SB. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical

applications. Chem Commun. 2007; 28: 2959-2961.
11. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci. 2009; 145: 83-96.
12. Dal Lago V, Franca OL, de Almeida GK, Kobarg J, Borba CM. Size-selective silver nanoparticles: future of biomedical devices with enhanced bactericidal properties. J Mater Chem, 2011; 21:12267-12273.
13. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against Gram-positive and Gram negative bacteria. Nanomedicine. 2012; 8: 37-45.
14. Syafiuddin A, Salmiati, Hadibarata T, Salim MR, Kueh ABH, Sari AA. A purely green synthesis of silver nanoparticles using Carica papaya, Manihot esculenta, and Morinda citrifolia: synthesis and antibacterial evaluations. Bioprocess Biosyst Eng. 2017; 40:1349- 1361. Doi: 10.1007/s00449-017-1793-z.
15. Zhang X-F, Liu Z-G, Shen W, Gurunathan S. Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci. 2016; 17(9): 1534
16. Pritam KD, Jatin K, Amit KD, Soumi S, Sunita S, Swati S, Piyush KG, Beom SK. Green synthesis of metallic nanoparticles: applications and limitations. Catalysts, 2021, 11(8): 902. http://doi.org/10.3390/catal11080902
17. Agnihotri S, Mukherji S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy. RSC Adv. 2014; 4: 3974-3983.
18. Raza M, Kanwal Z, Rauf A, Sabri A, Riaz S, Naseem S. Size- and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. Nanomaterials. 2016; 6: 1-15.
19. Mishra A, Tripathy SK, Yun S-I. Bio-synthesis of gold and silver nanoparticles from Candida guilliermondii and their antimicrobial effect against pathogenic bacteria. J Nanosci Nanotechnol. 2011; 11: 243-248.
20. Egwu IH, Iroha IR, Egwu-Ikechukwu MM, Peter IU, Nnabugwu CC, Ali CM et al.. Antimicrobial susceptibility pattern and molecular identification of Acinetobacter baumannii in Alex Ekwueme-Federal University Teaching Hospital, Abakaliki, Nigeria. Journal of pharmaceutical research international. 2021; 33(44B): 409-419. DOI: 10.9734/JPRI/2021/v33i44B32691.
21. Egwu IH, Egwu-Ikechukwu MM, Nnabugwu CC, Mustaha JK, Ali CM. Prevalence of uropathogens and its antimicrobial susceptibility in three mission hospitals in Abakaliki, Ebonyi State of Nigeria.Gomal J Med Sci. 2023; 21(1): 8-15. https://doi.org/1046903/gjms/21.01.1183.
22. Padalia RC, Verma RS, Chauhan AC. Changes in aroma profiles of 11 Indian Ocimum taxa during plant ontogeny. Acta Physiologiae Plantarum. 2013; 35(8): 2567-2587.
23. Abdullahi M. Phytochemical constituents and antimicrobial and grain protectant activities of Clove Basil (Ocimum gratissimum L.) grown in Niger. Journal of Plant Research. 2012; 2(1): 51-58.
24. Ashokkumar K, Vellaikumar S, Murugan M, Dhanya MK., Aiswarya S, Nimisha M. Chemical composition of Ocimum gratissimum essential oil from the South Western Ghats. India. Journal of Current Opinion in Crop Science. 2020; 1(1): 27-30.
25. Balavijayalakshmi J, Ramalakshmi V. Carica papaya peel mediated synthesis of silver nanoparticles and its antibacterial activity against human pathogens. Journal of Applied Research and Technology. 2017; 15: 413-422.
26. Sinthia G, Palanichamy M, Govindan, SS. Biosynthesis and characterization of silver nanoparticles using ginger spent and their antibacterial activity. Current Botany. 2021; 12: 174-179.
27. Achmad S, Salmiati, Tony H, Mohd RS, Ahmad BHK, Ajeng AS. A purely green synthesis of silver nanoparticles using Carica papaya, Manihot esculenta, and Morinda citrifolia: synthesis and antibacterial evaluations. Bioprocess

Biosyst Eng. 2017; 40:1349-1361.

28. European Committee for Antimicrobial Susceptibility Testing (EUCAST). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clinical Microbiology and Infection. 2000; 6: 509-515.
29. Olayinka AT, Onile BA. Bacterial use of antibiotics/antimicrobial agents, Nigerian medical practitioners. International Journal of Medicines and Medicinal Sciences. 2009; 33: 79-82.
30. Cheesebrough M. District laboratory practice in tropical countries. Cambridge University Press, New York 2010; Pp. 157-164.
31. Prakash B, Shukla R, Singh P, Mishra PK, Dubey NK, Kharwar RN. Efficacy of chemically characterized Ocimum gratissimum L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin B1 contamination of spices. Food Res. Int. 2011; 44: 385-390.
32. Baker C, Pradhan A. Synthesis and anti bacterial properties of silver nano particles. Journal of Nanoscience and Nano technology. 2005; 5(2): 244- 249.
33. Anuradha G, Sundar BS. Synthesis, Characterization, Antibacterial and Antifungal studies of Silver Nanoparticles from Ocimum gratissimum L. Int.J.Pharm Drug Anal. 2014; 2(9): 767-772
34. Nweze EI, Eze EE. Justification for the use of Ocimum gratissimum L in herbal medicine and its interaction with disc antibiotics. BMC Compl. Alternative Med. 2009; 9: 37.
35. Agholor K, Yaki LM, Abubakar I, Olusola LF, Zubairu R. Antibacterial activity of Ocimum gratissimum (Scent leaf) on some pathogenic gastrointestinal bacteria. African journal of microbiology research. 2018; 12 (40): 923-929. https://doi.org/10.5897/AJMR2018.8847
36. Ugbogu OC, Okezie E, Agi GO, Ibe C, Ekweogu CN, Ude VC, Uche ME, Nnanna RO, Ugbogu EA. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil(Ocimum gratissimumL.)