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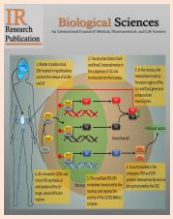

Comparative Antimicrobial Properties of a Consortium of *Nauclea latifolia* Sm. and *Ocimum gratissium* L. Extracts with Their CuO

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ABSTRACT

Over the years, several microorganisms have developed antibiotic resistance, rendering them impotent and otherwise useless. This research work is intended at exemplifying the consortium of crude extracts of *Nauclea latifolia* and *Ocimum gratissium* for antimicrobial potentials. Nanoparticles made from extracts were equated to the activities. Plant nanoparticles were created by combining powdered plant materials with copper oxide and suspending them in ethanol and distilled water, which were then screened to achieve crude extracts (CuO). The presence of bioactive machinery tartan in crude extracts. Agar well diffusion was betrothed to test antibacterial activity while the poisoned plate method was utilized to define antifungal potentials. Flavonoids, alkaloids, steroids, saponins, terpenoids, and tannins were discovered in plant extracts. At a dosage of 40 mg/mL, the aqueous crude extract outperformed the ethanolic extract, with the best inhibition zone (30) against *Bacillus megaterium*. The antimicrobial properties of the aqueous nanoparticle extract were stronger than those of the crude equivalent, antibacterial and antifungal inhibition zones were 36 mm at 40 mg/mL and 32 mm at 60 mg/mL respectively. According to the findings, aqueous extracts have stronger antibacterial activity than ethanolic extracts and nanoparticle created utilizing a syndicate of two plants has the potential to improve antimicrobial properties.

Introduction

The synthesis of complexes is usually done in one of two ways: top-down or bottom-up. Natural product researchers' attention has recently shifted to the use of alternative therapies in the diagnosis and treatment of infectious diseases (1). Nauclea latifolia, is an ageless multi-stemmed shrub or a tree, that grows up to an altitude of 200m. Infusions and decoctions of the barks and leaves are used to treat fever, diarrhea, abdominal disorders, and tropical diseases such as Malaria in West and South Africa. In Kano (Nigeria), *Nauclea latifolia* is used as a chewing stick and as a therapy against tuberculosis and stomach ache (2). Glycosides, saponins, alkaloids, and tannins are midst the phytoconstituents of this plant (3). Antimalarial and

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eczema treatments are made from crude extracts of the roots, leaves, and stem barks. *Ocimum gratissium* L., sometimes known as "Clove basil or African basil "a polymorphic branched, aromatic shrub nearly 0.5 to 3m tall (4) belonging to the family Lamiaceae, has been identified as a culinary herb with wide applications. The plant is mainly distributed in tropical areas and is native to South Asia, Africa, and various regions of South America. *Ocimum gratissium* is generally antimicrobial, antidiabetic, antidiarrheal, antimutagenic, and anticancerous in nature (5). *Ocimum gratissium* has been shown to have a number of active components that are beneficial to one's health (6). The influence of a plant on complex synthesis is determined by the secondary metabolite (7). The utilization of various plant extracts in the creation of nanoparticles has been described in several publications. Antimicrobial activity against pathogenic organisms is often enhanced by such combinations (8). As a result, the present study designed to synthesize copper complex utilizing a syndicate of *Nauclea latifolia* and *Ocimum gratissium*, as well as comparing the antibacterial properties of the nanoparticles to those of crude ethanolic and aqueous extracts of the plants.

Materials and methods

Extraction and collection of plant materials

The technique used by Taixiang et al (9) was employed in this research. The plants were rinsed with distilled water and dried, then ground into slight pieces with a mortar and pestle. Twenty grams of the pulverized sample was separately soaked in 100ml of distilled water and left for 48 hours, this serve as the aqueous extract. However, 20g of the powdered plant material was soaked in 100 ml of 95% ethanol for 48hrs at room temperature. These were kept on a rotary shaker at a revolution of 150rpm. The solution was filtered using sterile Whatman No.1 filter paper inserted in a funnel and the extract collected was stored in the refrigerator at 4oC until required for use. The resulting filtrate was concentrated by a rotary evaporator to obtain the crude extracts. The extracts were redissolved in their respective extractants to obtain a stock concentration of 200 mg/ml.

Biosynthesis of nanoparticles with Copper Oxide (CuO)

A copper oxide (CuO) solution (1 mM) was prepared and diversified separately with aqueous and ethanolic extracts of the combined effect of *Nauclea latifolia* and *Ocimum gratissium*. Pursuing the technique of Pirtarighat et al (10), the solution was shaken at room temperature for 4 hours before being decanted and dried for further analysis.

Preliminary phytochemical screening of plant extracts

Standard measures were used to determine the existence of the following plant constituents: alkaloids, steroids, terpenoids, tannins, flavonoids, and saponins (11).

Test for Flavonoid

1-5 drops of rigorous hydrochloric acid (HCl) were added to a minuscule quantity of ethanolic plant extract. The presence of flavonoids was indicated by the presence of yellow coloration in each extract.

Test for Alkaloids

In a test tube, 2.0 milliliters (ml) of extract were added, followed by 0.2 milliliters of dilute HCl and 1.0 milliliter (ml) of Meyer's reagent. The presence of an alkaloid was indicated by a yellowish coloration.

Test for Tannins

Five ml of plant extracts were placed in a test tube, followed by 2 ml of 5% ferric chloride (FeCl3) solution. The presence of tannins is indicated by a greenish-black precipitate.

Test for Steroids

Each sample received two (2) milliliters of acetic anhydride and two milliliters of hydrogen tetraoxosulphate (VI) acid (H2S04). In some samples, the color changed from violet to blue or green, indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of each extract was mixed with 2 ml of Chloroform before adding concentrated hydrogen tetraoxosulphate (VI) acid (H2S04) to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Saponin

In test tubes, 10 mL of distilled water was thoroughly mixed with the aqueous and ethanolic extracts. A few drops of olive oil were added to the foaming. The presence of saponin is indicated by the level of foam appearance.

Antimicrobial profile of the plant extracts and Nanoparticles

Collection and preservation of test microorganisms

Test organisms were selected based on those available, which subsequently covered a broad range/spectrum of microorganisms (Gram-positive and negative). Thus, seven bacteria isolates and four fungi were used as test organisms. Pure clinical isolates of bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus megaterium, Bacillus subtilis, Serratia marcescens,* and *Klebsiella pneumonia* were collected from Aminu Kano Teaching Hospital, Kano State, Nigeria, while *Bacillus subtilis, Pseudomonas aeruginosa*, and *Aspergillus niger* were collected from stock samples of the Microbiology Department Skyline University Nigeria. They were continuously sub-cultured in suited agars for purity and kept at 4℃ until needed.

Screening of crude extracts for antimicrobial properties

The agar well diffusion technique outlined by Ahmed et al. was used to test antibacterial activity (12). Using sterile swab sticks and the nutrient agar plate was inoculated with a 24-hour-old inoculation. At the center point of the inoculated plates, 5 mm wells were bored. The wells were filled with extracts (0.5 mL), the plates were then incubated at 370C for 24 hours and the diameter zone of inhibition was measured to the nearest millimeter.

This was also done with Candida albicans. The antifungal assay was performed using the poisoned plate technique described by Ahmed et al (12). Each test fungus had an agar plate that had been aged for 24 to 48 hours. Mycelial plugs were cut with a cork borer from the advancing precincts of these plates and pinned to the center of a solidified sterile potato dextrose agar (PDA) plate that had previously been cut open and its agar plug detached at the center. Radial growth was accurately measured after incubating the plates at room temperature for 24-48 hours. Hence, the antibacterial and antifungal activity of crude extracts and nanoparticles was tested.

Mechanisms of action of *Nauclea latifolia* and *Ocimum gratissium* on test isolates

Effect of plants extracts on the cell wall of test isolates

Formalin (0.2 ml) was introduced into the test tube, and 1 ml of each test culture with 1 ml of extract was distinctly add-on to the tubes. The contents were centrifuged for 15 minutes at 12,168x 103 g (MSE Minor 35). After centrifugation, the pellets of cells from the test tubes were re-suspended in 0.1 ml demineralized water. Smears were prepared on glass slides, dried and stained with diluted carbon fusion for 30 seconds, rinsed in water, air dried, and examined under the microscope and photomicrographs were taken at x400 (14).

Results

The aqueous and ethanolic extracts of the plant conjunction were evaluated and both extract types encapsulated the six assayed phytochemicals as shown in Table 1. The crude plant extracts' antibacterial activity was found to be higher in the aqueous extract than in the ethanolic extract. The highest activity (30 mm) was observed against *B. megaterium* at a concentration of 40 mg/mL, while the ethanolic crude extract in the assay demonstrated 12 mm against *B. subtilis* and *S. marcescens* at concentrations of 40 and 60 mg/mL respectively. The findings are summarized in Table 2. Table 3 presents the indulgent synthesis of the test fungi to crude extracts. The antibacterial assay exhibited a consistent drift. The aqueous crude extract inhibited the test fungi's mycelial growth better than the ethanolic extract, with the aqueous extract producing the highest antifungal effect (25 mm) at a concentration of 60 mg/mL and the ethanolic extract producing the highest activity (20 mm) at a concentration of 20 mg/mL. The antibacterial activity of nanoparticles synthesized from the two plants' mixture revealed that the aqueous extract was much more active than the ethanolic equivalent. The maximum activity against *B. megaterium* was ascertained to be 35 mm (Table 4). Table 5 shows the antifungal activity of the nanoparticles. Aqueous extracts had the highest activity (32 mm), while the ethanolic nanoparticles had the lowest activity (31 mm).

Table 1. Phytochemical screening consortium of *Nauclea latifolia* and *Ocimum gratissium* plant extracts.

Phytochemicals	Aqueous	Ethanol
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+
Steroids	+	+
Terpenoids	+	+
Tannins	+	+

Key: + Present, - Negative

The photomicrograph of treated slides with *Nauclea latifolia* and *Ocimum gratissium* plant extracts revealed that the cellular architecture of test isolates was altered when compared to untreated controls (control cells). Plate II clearly demonstrates this. (a) Cellular architecture of isolates treated with plant

extracts of *Nauclea latifolia* and *Ocimum gratissium*. (b) Left untreated (control cells).

components such as saponins, alkaloids, and so on. Previous research has revealed that some medicinal plants' antimicrobial activities are being studied all

Table 2. Antibacterial activity of Nauclea latifolia and Ocimum gratissium plant extracts

Organisms/ Plant Extract	Ethanolic Extract (mg/mL)				Aqueous Extract (mg/mL)			
	20	40	60	80	20	40	60	80
Bacillus subtilis	10	12	7	6	20	18	19	24
Bacillus megaterium	8	5	10	5	24	30	16	11
Escherichia coli	4	8	4	5	19	27	29	16
Klebsiella pneumonia	12	13	16	18	2	5	3	6
Pseudomonas aeruginosa	4	3	2	8	11	13	12	16
Serratia marcescens	10	10	12	7	16	13	21	16
Staphylococcus aureus	3	8	4	6	12	13	16	18

Table 3. Antifungal activity of Nauclea latifolia and Ocimum gratissium plant extracts

Organisms/ Plant Extract	Ethanolic Extract (mg/mL)				Aqueous Extract (mg/mL)			
	20	40	60	80	20	40	60	80
Cryptococcus neoformans	13	7	12	16	9	13	7	17
Penicillium chrysogenum	15	-	21	17	14	20	7	15
Candida albicans	20	12	16	19	7	16	25	22
Aspergillus niger	13	1	-	-	14	11	6	18

Table 4. Antibacterial activity of *Nauclea latifolia* and *Ocimum gratissium* nanoparticles

Organisms/ Plant Extract	Ethanolic Extract (mg/mL)				Aqueous Extract (mg/mL)			
	20	40	60	80	20	40	60	80
Bacillus subtilis	15	27	19	14	15	26	18	24
Bacillus megaterium	5	20	27	17	27	36	23	17
Escherichia coli	24	19	14	18	23	22	18	12
Klebsiella pneumonia	10	14	16	19	7	11	22	10
Pseudomonas aeruginosa	11	15	15	23	2	7	28	5
Serratia marcescens	18	11	7	15	8	26	21	12
Staphylococcus aureus	18	24	19	12	16	9	20	18

Table 5. Antifungal activity of *Nauclea latifolia* and *Ocimum gratissium* nanoparticles

Organisms/ Plant Extract	Ethanolic Extract (mg/mL)				Aqueous Extract (mg/mL)			
	20	40	60	80	20	40	60	80
Cryptococcus neoformans	13	15	19	15	17	4	15	17
Penicillium chrysogenum	17	13	29	14	15	23	14	16
Candida albicans	12	19	26	17	22	3	32	26
Aspergillus niger	28	18	13	15	18	11	14	24

Discussion

The presence of alkaloids, flavonoids, steroids, saponins, tannins and terpenoids was discovered in crude aqueous and ethanolic extracts of *Nauclea latifolia* and *Ocimum gratissium*. These compound groups may have been accountable for the antimicrobial activity observed in the current research work. The previous study revealed that alcohol was used for abstraction with the utmost medicinal plants in order to attain a pure and efficient compound (15). Plant extracts contain plant

over the world. It has also been stated that about 80% of plant extracts are used as herbal drugs across the globe (16). Mabhiza et al (17) demonstrated the antibacterial activity of *Vernonia adoensis* and *Callistemon citrinus* alkaloid extracts against *P. aeruginosa* and *S. aureus*, as well as inhibition of ATP-dependent transport of compounds via microbial cell membranes. Steroids have been shown to have a wide range of antibacterial activity. Tannins can also act as a siderophore, removing iron from the medium and making it inaccessible to organisms. Table 2 shows the antibacterial activity of various *Nauclea latifolia*

and *Ocimum gratissium* extracts against the organisms at various concentrations. The diameter sector of inhibition on nutrient agar plates revealed that the aqueous extract of the combined plants

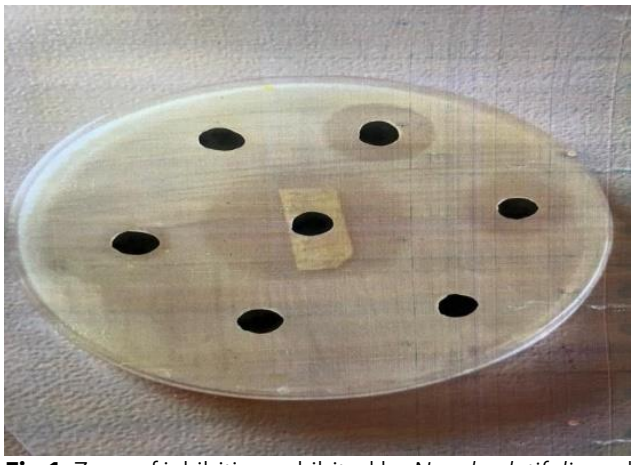

Fig 1. Zone of inhibition exhibited by *Nauclea latifolia* and *Ocimum gratissium* plant extracts against Klebsiella pneumonia.

components, and their pollution significantly suppresses host defenses (19).

These factors could be blamed for A. niger's resistance in this study. The antifungal assay results showed that the nanoparticles had excellent antifungal sensitivity against A. niger. This could be due to the complex's use of metallic oxides (CuO). Plant nanoparticles' activity, according to the literature, is due to their great surface-to-volume ratio, which makes them highly reactive. This is consistent with Prabhu and Poulose (20)'s discovery that nanoparticles can conform to and infiltrate the microbial cell wall, disrupting membrane fluidity (21). The plant extracts' mechanisms of action are thought to be several activities of the various phytoconstituents in disrupting the normal permeability of the test cells, making them susceptible to cell lysis and architectural injury. Furthermore, these multiplexes can pass through microbial cell membranes (22), allowing them to enter target cells.

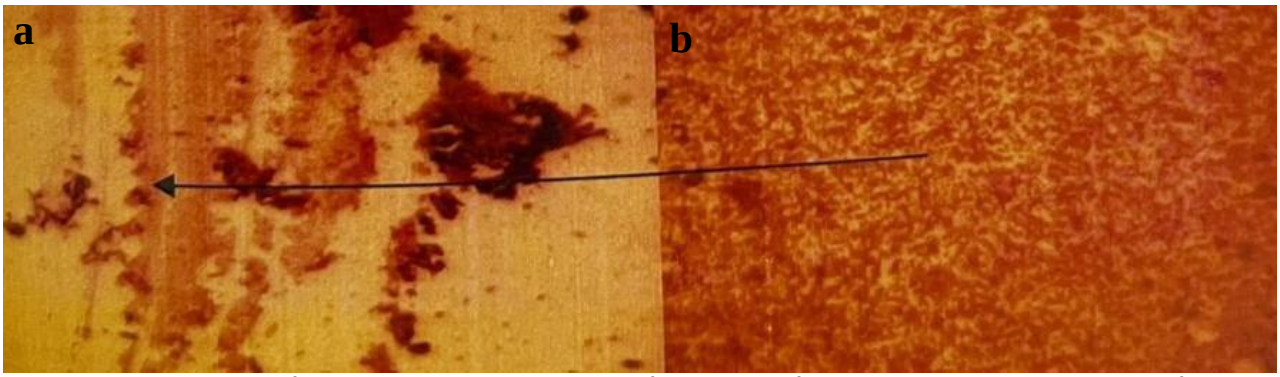

Fig 2. Photomicrograph of distorted cellular architecture of as a result of exposure to aqueous Nauclea latifolia and Ocimum gratissium plant extracts and the control (X400). (a) Cellular architecture of Pseudomonas aeruginosa treated with aqueous extract of Nauclea latifolia and Ocimum gratissium. (b) Untreated (Control).

was more effective against the organisms than the ethanolic extract. Thus, the benefits of the extract from this research substantiate the use of the two plants in indigenous folkloric medicine. The discovered activity of the plant's aqueous extract could be related to water polarity. Water is a versatile, remarkably polar solvent capable of solubilizing a wide range of polar phytoconstituents, resulting in extractions (18). This is similar to the findings of Ahmed et al (12), who discovered that water is an energetic solvent for secondary metabolite extraction from flowers. According to antibacterial and antifungal assays, the crude extracts (ethanolic and aqueous) of the two plants were effective against all of the test isolates except Aspergillus niger, which became resistant to the plants' ethanolic crude extracts. According to the literature, A. niger encompasses some malignant elements that contribute to its pathogenicity. Many metabolites produced by A. niger inhibit phagocytosis and opsonization. Furthermore, Aspergillus species can bind to specific host tissue

Conclusion

Plant extracts of *Nauclea latifolia* and *Ocimum gratissium* have been shown to be synthesized with antimicrobial CuO nanoparticles. The antimicrobial activity of aqueous extracts was found to be greater than that of ethanolic extracts. The synthesis of the two plants' nanoparticles may improve antimicrobial effectiveness. It is recommended, however, that further investigations should be made about the test isolates and plant in order to reveal its other uses as well as likely toxicity.

Contribution of authors

Mustapha Abdulsalam Participated in all experiments, coordinated the antimicrobial profile of the plants extracts and nanoparticle; screening of crude extract and contributed to the writing of the manuscript. Bashir Bolaji Tiamiyu Participated in all experiments, coordinated the extraction and

collection of plant material; mechanism of action and also contributed to the writing of the manuscript. Ibrahim Abdulrazaq Coordinated the laboratory work, biosynthesis of nanoparticle with CuO and phytochemical screening. Salam Olaitan Lateefat Designed the research plan, proofread the manuscript and organized the study.

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Conflict of interest

The authors have declared no conflicts of interest.

References

1. Urits, I., Schwartz, R. H., Orhurhu, V., Maganty, N. V., Reilly, B. T., Patel, P. M., Wie, C., Kaye, A. D., Mancuso, K. F., Kaye, A. J., Viswanath, O. (2021). A Comprehensive Review of Alternative Therapies for the Management of Chronic Pain Patients: Acupuncture, Tai Chi, Osteopathic Manipulative Medicine, and Chiropractic Care. Advances in Therapy, 38(1):76-89.
2. Iheagwam, F. N., Israel, E. N., Kayode, K. O., DeCampos, C., Ogunlana, O. O., Chinedu, S. N. (2020). *Nauclea latifolia* Sm. Leaf Extracts Extenuates Free Radicals, Inflammation, and Diabetes-Linked Enzymes. Oxidative Medicine and Cellular Longevity, 2020:5612486.
3. Oke, E. O., Okolo, B. I., Adeyi, O., Agbede, O. O. , Nnaji, P, C., Adeyi, J. A. , Osoh, K. A., Ude. C. J. (2020). Black-box modeling, bi-objective optimization, and ASPEN batch simulation of phenolic compound extraction from *Nauclea latifolia* root. Heliyon, 7(1): e05856.
4. Ugbogu, O. C., Emmanuel, O., Agi, G. O., Ibe, C., Ekweogu, C. N., Ude, V. C., Uche, M. E., Nnanna, R. O., Ugbogu, E. A. (2021). A review of the traditional uses, phytochemistry, and pharmacological activities of clove basil (Ocimum gratissimum L.). Heliyon, 7(11): e08404.
5. Zhan, Y., An, X., Wang, S., Sun, M., Zhou, H. (2020). Basil polysaccharides: A review on extraction, bioactivities and pharmacological applications. Bioorganic and Medicinal Chemistry, 28(1):115179.
6. Balaji, R., Ravichandiran, K., Tanuja and Parani, M. (2021). The complete chloroplast genome of Ocimum gratissimum from India - a medicinal plant in the Lamiaceae. Mitochondrial DNA Part B Resources, 6(3):948-950.
7. Kozsup, M., Zhou, X., Farkas, E., Cs Bényei, A., Bonnet, S., Patonay, T., Kónya, K., Buglyó, P. (2021). Synthesis, characterization and cytotoxicity studies of Co(III)-flavonolato complexes. Journal of Inorganic Biochemistry, 217:111382.
8. Eilers, A., Witt, S and Walter, J. (2020). Aptamer-Modified Nanoparticles in Medical Applications. Advances in Biochemical Engineering/ Biotechnology. 174:161-193.
9. Chen, T., Qi, Y., Wang, L and Li, C. (2021). First Report of Leaf Spot Disease on Fagopyrum esculentum caused by Bipolaris zeae in China. Plant Diseases. doi: 10.1094/PDIS-01-21-0020-PDN. Online ahead of print.
10. Pirtarighat, S., Ghannadnia, M and Baghshahi, S. (2019). Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. Journal of Nanostructure in Chemistry, 9(1):1-9.
11. Ahmed, R. N., Sani, A., Ajiboye, A. E., Gambari-Ambali, R. O., Ezekiel, Ku. (2013). Sensitivity of three gastrointestinal organisms to aqueous extract of leaf of Ocimum gratissimum. Nigeria Journal of Pure and Applied Sciences, 26:2460- 2469.
12. Ahmed, R. N., Sani, A., Igunnugbemi, O. O. (2009). Antifungal profiles of extracts of Vitellaria paradoxa (Shea-Butter) bark. Ethnobotanical leaflets, 13:679-688.
13. Karim, R.(2020). Identification of phytochemicals from North African plants for treating Alzheimer's diseases and of their molecular targets by in Silico network pharmacology approach. Journal of Traditional and Complementary Medicine, 11(3):268-278.
14. Soliman, E. S., Hamad, R.T and Abdallah, M. S. (2021). Preventive antimicrobial action and tissue architecture ameliorations of Bacillus subtilis in challenged broilers. Veterinary World, 14(2):523-536.
15. Ștefănescu, R., Tero-Vescan, A., Negroiu, A., Aurică, E and Camil-Eugen, Vari, C. E. (2020). A Comprehensive Review of the Phytochemical, Pharmacological, and Toxicological Properties of Tribulus terrestris L. Biomolecules, 10(5):752.

16. Tomee, J. F and Kauffman, H. F. (2000). Putative virulence factors of Aspergillus fumigatus. Clinical and Experimental Allergy. 30(4):476-484.
17. Mabhiza, D., Chitemerere, T and Mukanganyama, S. (2016). Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa. International Journal of Medicinal Chemistry; 2016:6304163.
18. Bamigboye, Oluwaseyi Mercy., Ahmed, R. N. (2019). Comparative Antimicrobial Activities of a Consortium of Vernonia amygdalinaand Amaranthus hybridus Extracts With Their CuO Nanoparticle Complexes. International Journal of Medicinal Review, 6(1):31–34.
19. Gazi, U , Rosas, M., Singh, S., Heinsbroek, S., Haq I., Johnson, S., Brown, B. G., Williams, D. L., Taylor, P. R., Martinez-Pomares, L. (2011). Fungal recognition enhances mannose receptor shedding through dectin-1 engagement. Journal of Biological Chemistry, 286(10):7822-7829.
20. Prabhu, S and Poulose, E. K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letter. 2(1):32.
21. Sabnis, A., Katheryn, L. H ., Klöckner, A., Becce, M., Lindsay, Evans, L. E., Furniss, R. C., Mavridou, D. A., Murphy, R., Stevens, M. M., Davies, J.C., Larrouy-Maumus, J. G., Clarke, T. B and A. M. (2021). Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane. Elife,10: e65836.
22. Ellis, B. M, Babele, P. K., May, J. C., Johnson, C. H., Pfleger, B. F., Young, J. D and McLean, J. A. (2021). Accelerating strain phenotyping with desorption electrospray ionization-imaging mass spectrometry and untargeted analysis of intact microbial colonies. Proceeding of the National Academy of Sciences USA, 118(49): e2109633118.