Insights into Comparative Antimicrobial Efficacies of Synthetic and Organic Agents: The Case of ZnS Nanoparticles and Zingiber officinale *Rosc.*

O. F. Obidi, A. O. Nejo, R. A. Ayeni & N. Revaprasadu

JOM
The Journal of The Minerals, Metals & Materials Society (TMS)

ISSN 1047-4838
Volume 70
Number 6

JOM (2018) 70:982-987
DOI 10.1007/s11837-018-2816-1

Springer

JOM, Vol. 70, No. 6, 2018
https://doi.org/10.1007/s11837-018-2816-1

TECHNICAL COMMUNICATION

Insights into Comparative Antimicrobial Efficacies of Synthetic and Organic Agents: The Case of ZnS Nanoparticles and *Zingiber officinale* Rosc.

O.F. OBIDI,[1,4] A.O. NEJO,[2] R.A. AYENI,[1] and N. REVAPRASADU[3]

1.—Department of Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria. 2.—Department of Chemistry, Faculty of Science, University of Lagos, Lagos, Nigeria. 3.—Department of Chemistry, University of Zululand, Kwadlangezwa, South Africa. 4.—e-mail: laideob@yahoo.com

The differences among the antimicrobial activities of synthetic nanoparticles (NPs), organic agents and conventional antibiotics against human pathogens are little known. We compared the antimicrobial activities of aqueous, ethanol and ethyl acetate extracts of *Zingiber officinale* rhizomes with ZnS NPs and tetracycline/nystatin using agar-diffusion techniques. Transmission electron microscopy (TEM), Fourier transform infrared (FTIR) and ultraviolet spectroscopy were used to characterize ZnS NPs. At 100 mg/ml, ethanol and ethyl acetate extract inhibited *Acinetobacter baumannii, Salmonella typhimurium, Enterococcus faecium, Shigella flexneri, Klebsiella pneumoniae, Staphylococcus epidermidis and Candida albicans* with zones of inhibition (ZOI) ranging between 0–42 mm and 0–39 mm, respectively. *Candida albicans* had a remarkable ZOI of 42 mm and 22 mm from ethanol and ZnS NPs compared with 20 mm from conventional nystatin. TEM and FTIR revealed spherically shaped polydispersed NPs with particle size of 12.5 nm and the role of banana peel extracts in ZnS NPs synthesis. Organic and synthetic NPs proved potential alternatives to conventional antimicrobial agents.

INTRODUCTION

The use of plants as traditional remedies for various infections in many parts of the world is an age-old practice and well documented.[1–3] Traditional medical practice has been embraced because of its efficacy and contribution to health care.[4–6] The development of microbial resistance to conventional antibiotics has also promoted the current use of medicinal plants as antimicrobial agents.[7–9] Plants offer great potential as antimicrobial agents because they contain a variety of active chemical substances that cure infections.[10–16] Herbs, rhizomes, bark and leaves of various plants are employed to treat several infections ranging from the common cold to severe infections such as gonorrhea.[17] They can be in the form of powders or liquids mixtures and may be raw or boiled, applied as ointments, liniments, and incisions.[18] About 80% of the world population relies on botanical preparations as medicines to meet their health needs.[19] Where poisoning has been reported, it has been linked to misidentification, incorrect preparation and administration, interaction with conventional drugs, the method of extraction and presence of heavy metals or pesticides. All these may greatly interfere with quality and safety (5). However, the adverse effects of most herbal drugs are relatively less frequent when the drugs are used properly compared with synthetic drugs.[5,6] *Zingiber officinale* Rosc. is one of the most well-known species of the Zingiberaceae family because of its antimicrobial and medicinal values.[20] Ginger has provided an effective and safe remedy for ailments with microbial origins for 2500 years.[21–23] It is native to Southern Asia, but is now extensively cultivated in India, China, Japan, Indonesia and Nigeria.[24] The antimicrobial properties of ginger, which may depend on the mode and solvent of extraction,[25,26] have been linked to its extremely high levels of phytochemicals. Important secondary metabolites present in ginger are curcumene, non-volatile hydroxyaryl compounds, volatile sesquiterpenes and mono-terpenoids.[27] Eleazu

(Published online March 23, 2018)

et al.[28] reported the presence of saponins, alkaloids, flavonoids, tannins and cyanogenic glycosides in ginger. Interest in nanoparticles, which are quite novel and mostly synthetic, has been generated in recent years to combat the emerging resistance to antimicrobials. Nanoparticles have a simple structure and characteristic physical, chemical and biological properties that distinguish them from those of the bulk materials. Intensive experiments and studies have revealed that the nanoparticles of MgO, CaO and ZnO[29,30] possess potent antimicrobial properties when tested against various Gram-positive and -negative organisms. ZnS has numerous applications to its credit such as in photoconductors, solar cells, transducers, optical coatings and light-emitting materials. However, information on the antimicrobial properties of ZnS NPs is either scanty or nonexistent. ZnS nanocrystals have been considered a good candidate to replace the heavy metals contained in semiconductor nanoparticles.[31] This is because they are non-toxic and contains zinc, which is essential for human consumption at 10-15 mg/day. This study compares the in vitro antimicrobial activities of ginger with inorganic ZnS NPs. To the best of our knowledge, this is the first report of such comparisons between organic and inorganic antimicrobial agents.

MATERIALS AND METHODS

Plant Materials and Extract Preparation

Healthy rhizomes of ginger (*Zingiber officinale* Rosc.) were purchased at Sabo market, Ikorodu, Lagos, Nigeria. They were sun-dried for 7 days and ground into fine powder using an electric grinder (Qlink, People's Republic of China). The dried (100 g) powdered mass obtained was stored in sterile bottles at room temperature and used for the extractions. Twenty grams of the powdered plant material was dissolved in 100 ml each of distilled water, 95% ethanol and ethyl acetate in separate 500-ml sterile conical flasks at room temperature. The mixture was stirred, covered, allowed to stand for 24 h and subsequently filtered to obtain a crude extract using sterile Whatman no. 1 filter paper. The filtrate was concentrated to 20 ml on a water bath (Uniscope SM 801A Laboratory water bath, UK) and evaporated to dryness at room temperature as described by Malu et al.[32] The filtrates were then stored in separate bottles at room temperature until needed. The extracts were transferred into clean bottles and various volumes of the different solvents were added onto the extract to give final concentrations of 100 mg/ml, 90 mg/ml, 80 mg/ml, 70 mg/ml and 60 mg/ml, respectively.

Phytochemical Screening of Plant Extracts

The prepared extracts were qualitatively screened for the presence of tannins, alkaloids, saponins, flavonoids, phlobatannins and cardiac glycosides using the standard phytochemical tests described by Trease and Evans.[33]

Microbial Cultures

The test microorganisms used comprise four Gram-negative bacteria [*Acinetobacter baumannii* (MG 01289518-1), *Klebsiella pneumoniae* (ATCC 8308), *Shigella flexneri* (ATCC 12022) and *Salmonella typhimurium* (ATCC 13311)], two Gram-positive bacteria [*Staphylococcus epidermidis* (ATCC 12228) and *Enterococcus faecium* (ATCC 700221)] and a fungus [*Candida albicans* (ATCC 10231)]. They were obtained from the stock culture collection of the department of Microbiology, Faculty of Science, University of Lagos, Nigeria. The microorganisms were cultured on agar slants, stored at 4°C and subcultured at intervals.

Culture Medium and Inoculum Preparation

Cell suspensions were obtained by subculturing onto fresh plates of Mueller–Hinton agar (MHA) (Biomark, India) incubated at 37°C for 24 h and Sabouraud dextrose agar (SDA) (SRL, India) at room temperature for 3–7 days for bacteria and fungi, respectively. Subsequently, the inoculum was prepared by the colony suspension method. Colonies were touched with a loop and transferred to sterile normal saline. The suspension was adjusted to give a final concentration of 10^8 cells equivalent to that of 0.5 McFarland standard and plated out.

Banana Extract Preparation

Banana peels were washed and boiled in distilled water for 30 min at 90°C. One hundred twenty grams was crushed in 100 ml distilled water and filtered. The filtrate was treated with an equal volume of acetone and centrifuged at 1500 rpm for 5 min, and the precipitate was suspended in distilled water and stored at 4°C.

Synthesis of ZnS Nanoparticles

Sulfur (0.21 g), banana extract (6 ml) and $ZnCl_2$ (0. 9 g) were introduced into a round-bottom flask. The reaction mixture was then heated in a reflux setup for 6 h under nitrogen atmosphere. Acetone (10 ml) was added to the product obtained from the reflux and centrifuged for 15 min at 1500 rpm. The mixture was decanted and dried. The ZnS nanoparticles were subsequently characterized.

Characterization of Banana-capped ZnS NPs Using TEM, UV and FTIR

The morphology and particle sizes of the samples were characterized by a JEOL 1010 TEM with an accelerating voltage of 100 kV, Megaview III camera and Soft Imaging Solutions iTEM software. A T90+ UV/Vis spectrophotometer was used to carry out optical measurements by continuous scanning from 190 nm to 800 nm. The samples were placed in silica cuvettes (1-cm path length) using distilled water as reference solvent. The functional groups in

the synthesized solution were analyzed by FTIR spectroscopy. These measurements were carried out using a Bruker infrared spectrophotometer with a wavelength range of 4000–400 nm. The results were compared for shifts in functional peaks.

Antimicrobial Susceptibility Testing

The antimicrobial activity of aqueous 95% ethanol and ethyl acetate extracts of ginger and ZnS NPs were evaluated using the agar diffusion method. Standardized inoculum (0.1 ml) of test organisms was seeded uniformly by means of a sterile swab dipped in the suspension and streaked on MHA plates[34] for bacterial species and SDA plates for fungi. Wells of 7 mm diameter were punched onto the seeded plates using a sterile cork borer and filled with 0.1 ml of the extracts and ZnS NPs. The plates were allowed to stabilize for 1 h for proper diffusion of the antimicrobial agents and absorption of excess fluid before incubating aerobically at 37°C for 24 h and at room temperature for 48 h for bacteria and fungi, respectively. Standard antibiotic (tetracycline) and antifungal agents (Nystatin) were used as positive controls for bacterial species and fungi, respectively, while solvents used for ginger extraction (distilled water, 95% ethanol and ethyl acetate) were used as negative controls. Antimicrobial activity was evaluated by measuring the clear halo zones of inhibition around the wells after incubation in millimeters. An antimicrobial agent was categorized as active when the diameter of the inhibition was ≥ 8 mm.[35]

Determination of Minimum Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC)

The MIC values of the extracts and ZnS NPs were determined by the broth dilution method. Test tubes (18 × 150 ml) containing Mueller–Hinton broth (MHB) and Sabouraud dextrose broth (SDB) were inoculated with inocula from fresh overnight colonies of MHA (bacteria) and SDA (fungi). The suspensions were adjusted to the 0.5 McFarland turbidity standard. Stock extract of 200 mg/ml was prepared with the individual extract to be tested. Subsequently, serial two-fold dilutions were made in a concentration range from 100 mg/ml to 3.13 mg/ml. The lowest concentration of each extract of ginger and ZnS NPs showing a clear inhibition of visible growth of the test organism with no turbidity compared with the negative control tubes was taken as the MIC. The MBC and MFC were determined by subculturing from the broth dilution of the MIC test onto a fresh MHA and SDA solid medium using a sterile inoculating loop. The plates were incubated at 37°C for 24 h for bacteria and at room temperature for 24–48 h for fungi. The highest dilution and lowest concentration that yielded no single microbial colony on a solid medium was taken as the MBC and MFC as described by Igwo-Ezikpe et al.[36] All experiments were performed in duplicate, expressed as mean values and two-way analysis of variance (ANOVA) done at a 5% level of significance.

RESULTS

Phytochemical Constituents

The results of the phytochemical constituents of the aqueous extract (AE), ethanol extract (EE) and ethyl acetate extracts (EAE) of ginger showed the presence of tannins, flavonoids, alkaloids, cardiac glycosides and phlobatannins but no saponins in the EE and EAE. The AE had all the bioactive constituents except alkaloids.

Antibacterial Activity

Table I shows the ZOI of different concentrations of the ginger extracts. Results indicated that EE and EAE displayed antimicrobial activity while AE did not show any antimicrobial activity on test microorganisms. The EE of ginger showed a ZOI of 13 mm, 12 mm, 16 mm, 16 mm, 22 mm, 0 mm, and 42 mm at 100 mg/ml while the EAE of ginger exhibited a ZOI of 22 mm, 33 mm, 20 mm, 22 mm, 21 mm, 0 mm, and 39 mm on *A. baumanii, S. typhimurium, E. faecium, S. flexneri, K. peumoniae, S. epidermidis and C. albicans*, respectively. The EE showed a maximum ZOI of 42 mm at 100 mg/ml on *C. albicans* and a minimum ZOI of 7 mm at 70 mg/ml on *S. typhymurium*. All extracts were ineffective against *S. epidermidis* at all concentrations tested (Table I). Data obtained indicated that the EAE of ginger exhibited a maximum ZOI of 33 mm at 100 mg/ml on *S. typhimurium* and a minimum ZOI of 8 mm at 70 mg/ml and 60 mg/ml on *S. typhimurium* among the test bacteria. It had a maximum ZOI of 39 mm at 100 mg/ml and minimum ZOI of 32 mm at 60 mg/ml on *C. albicans*. Comparatively, ZnS NPs had a ZOI of 19 mm, 21 mm, 19 mm, 19 mm, 8 mm, 20 mm, and 22 mm at 100 mg/ml on *A. baumanii, S. typhimurium, E. faecium, S. flexneri, K. peumoniae, S. epidermidis and C. albicans*, respectively. The highest antimicrobial activity was observed in *C. albicans* with a ZOI of 22 mm and the lowest on *K. pneumoniae* with a minimum ZOI of 7 mm at 70 mg/ml and 60 mg/ml (Table I). The remarkable activity of the ZnS NPs on *S. epidermidis* is however exceptional. The EE and EAE of ginger did not produce any ZOI on *S. epidermidis*. However, a ZOI of 18 mm, 18 mm, 18 mm, 19 mm, and 20 mm was observed at increasing concentrations of the ZnS NPs (Table I). At 100 mg/ml the ZnS NPs with a ZOI of 22 mm also performed better than conventional Nystatin with a ZOI of 20 mm on *C. albicans*. The MIC assay (Table II) showed that a 95% EE had greater activity toward *K. pneumoniae and C. albicans* with an MIC of 25 mg/ml and less activity toward *A. baumannii*, *S. typhimurium*, *E. faecium*, *S. flexneri* and

Table I. Zone of Inhibition (ZOI) of ginger extracts, ZnS NPs and conventional tetracycline/nystatin against test organisms

	Zone of inhibition (mm)											
	95% ethanol extract						Ethyl acetate extract					
Test organisms	**100 mg/mL**	**90 mg/mL**	**80 mg/mL**	**70 mg/mL**	**60 mg/mL**	**95% Ethanol**	**100 mg/mL**	**90 mg/mL**	**80 mg/mL**	**70 mg/mL**	**60 mg/mL**	**Ethyl acetate**
Acinetobacter baumannii	13	11	11	9	8	0	22	14	13	12	11	0
Salmonella typhimurium	12	10	8	7	0	0	33	32	17	8	8	0
Enterococcus faecium	16	10	8	0	0	0	20	14	11	11	10	0
Shigella flexneri	16	14	12	0	0	0	22	16	0	0	0	0
Klebsiella pneumoniae	22	20	18	18	14	22	21	20	16	11	9	18
Staphylococcus epidermidis	0	0	0	0	0	0	0	0	0	0	0	0
Candida albicans	42	40	38	37	35	35	39	39	36	34	33	32

	Zone of inhibition (mm)															
	Distilled water extract						ZnS NPs					Tetracycline/nystatin				
Test organisms	**100 mg/mL**	**90 mg/mL**	**80 mg/mL**	**70 mg/mL**	**60 mg/mL**	**D/Water**	**100 mg/mL**	**90 mg/mL**	**80 mg/mL**	**70 mg/mL**	**60 mg/mL**	**100 mg/mL**	**90 mg/mL**	**80 mg/mL**	**70 mg/mL**	**60 mg/mL**
Acinetobacter baumannii	0	0	0	0	0	0	19	18	14	16	14	45	43	42	39	37
Salmonella typhimurium	0	0	0	0	0	0	21	19	16	16	12	43	41	41	40	39
Enterococcus faecium	0	0	0	0	0	0	19	18	10	10	8	41	41	40	30	36
Shigella flexneri	0	0	0	0	0	0	19	17	12	12	10	40	38	36	35	33
Klebsiella pneumoniae	0	0	0	0	0	0	8	8	7	7	7	44	43	43	40	39
Staphylococcus epidermidis	0	0	0	0	0	0	20	19	18	18	18	42	41	41	39	38
Candida albicans	0	0	0	0	0	0	22	17	15	13	12	20	18	17	17	15

Table II. Minimum inhibitory concentrations (MICs) of *Zingiber officinale* extracts, ZnS NPs and Conventional tetracycline/nystatin

Antimicrobial agents	*Acinetobacter baumanni* (mg/mL)	*Salmonella typhimurium* (mg/mL)	*Enterococcus Faecium* (mg/mL)	*Shigella flexneri* (mg/mL)	*Klebsiella pneumoniae* (mg/mL)	*Staphyloccus epidermidis* (mg/mL)	*Candida albicans* (mg/mL)
95% ethanol extract	50	50	50	50	25	50	25
Ethyl acetate	100	25	100	50	50	50	50
Distilled water	> 100	> 100	> 100	> 100	> 100	> 100	> 100
ZnS NPs	25	25	25	25	25	25	50
Tetracycline	3.13	3.13	3.13	3.13	3.13	3.13	–
Nystatin	–	–	–	–	–	–	> 100

S. epidermidis with an MIC of 50 mg/ml. EAE had the highest activity on *K. pneumoniae* with an MIC of 12.5 mg/ml. This was followed by *S. typhimurium* (MIC 25 mg/ml) and then *S. flexneri, S. epidermidis* and *C. albicans* (MIC 50 mg/ml). The least activity of EAE was on *A. baumanii* and *E. faecium* (MIC 100 mg/ml). On the other hand, ZnS NPs performed appreciably well with an MIC of 25 mg/ml for all the organisms and 50 mg/ml on *C. albicans*. Nystatin had a > 100 mg/ml MIC for *C. albicans* while tetracycline had an MIC < 13.3 for all the bacteria tested (Table II). The MBC for ethanol extract was 25 mg/ml for *C. albicans* and 50 mg/ml for *Shigella flexneri*, *Klebsiella* and *Salmonella typhimurium*. The MBC/MFC of ZnS NPs and Nystatin was > 100 mg/ml for all organisms tested (Supplemental Table I) while the MBC of tetracycline was < 3.13 for *S. typhimurium, S. flexneri, K. pneumoniae and S. epidermidis.* Statistical analysis shows *Zingiber officinale* extracts had a significant effect ($F = 19.46$, $p < 0.0001$) on MICs of organisms.

TEM

The TEM image of the banana-capped ZnS nanoparticles and histogram showed spherically shaped polydispersed nanoparticle (see supplemental material Fig. 1). The sulfide nanoparticles have few aggregates, and an average particle size of 12.5 nm was obtained from the statistical analysis of the Gatan Digital Micrograph and OrginPro 8.5 software.

UV

The UV spectra of banana-capped ZnS nanoparticles show the optical band gap energy was 3.27 eV compared with the bulk ZnS of 337 nm (3.68 eV)[37] (Supplemental Fig. 2b). A red shift indicated an increase in either the size or aggregation of ZnSNPs in BPE; the TEM confirms the same.

FTIR

The FTIR spectra of banana-capped ZnS NPs are recorded in the range 4000–400 cm^{-1} (Supplemental Fig. 2). Compared with the spectra given by the pure banana peel extracts,[38] it was observed that for the banana-capped, pH 11, ZnS nanoparticles, there were shifts in peaks from 3411–3425 cm^{-1}, 1637–1611 cm^{-1} and 1386–1344 cm^{-1}. This indicates the involvement of carboxyl, amine and hydroxyl groups in the formation of ZnS NPs. The 2932 cm^{-1} peak disappeared in the spectra, suggesting its involvement in reduction during the nanoparticle synthesis.

DISCUSSION

The present study revealed that ginger has potent antimicrobial properties against the tested microbes. The presence of phytochemical constituents, which are plant secondary metabolites and known to inhibit microorganisms,[39,40] probably contributed to the antimicrobial action observed. Ginger has been reported to prevent vomiting and stop diarrhea and is traditionally useful in the treatment of infectious diseases.[41] The EE and EAE of the ginger against Gram-positive and -negative bacterial isolates suggest that the EE and EAE contain components that may have disrupted the membrane and/or genetic make-up of the organisms and therefore had a better antimicrobial effect than the AE, which showed no antimicrobial effect. This is in line with the results of Onyeagba et al.[18] who showed that alcohol was a better solvent for extraction of antimicrobially active substances compared with water. The result also showed that ginger has antimicrobial properties against a wide range of organisms. This corroborates the report of Gur et al.[41] showing that ginger exhibited valuable antimicrobial activities. Furthermore, the comparison of bioactive compounds in ginger extracts suggested that the EE and EAE of ginger root could be potent against infections while the AE extract of ginger roots could be ineffective. The increased antimicrobial activity of banana-capped ZnS NPs could be due to the synergistic interaction between the banana and ZnS nanoparticles. Banana peels have been found to contain secondary metabolites such as flavonoids, which have been shown to have antimicrobial activity.[42] Previous studies have reported that ZnS nanoparticles discharge ions that react with thiol groups in the protein cell membrane. This phenomenon disrupts the cell functions and eventually kills the microorganisms.[43–45]

CONCLUSION

The study reveals the antimicrobial potentials of banana-capped ZnS NPs, which are little known compared with its other properties, which are well reported, and suggests the potentials of ginger as an effective antimicrobial agent that can replace the conventional antimicrobial agents in combating resistant organisms.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (https://doi.org/10.1007/s11837-018-2816-1) contains supplementary material, which is available to authorized users.

REFERENCES

1. A. Nick, T. Rali, and O. Sticher, *J. Ethnopharmacol.* 49, 147 (1995).
2. K.O. Akinyemi, O. Oladapo, C.E. Okwara, C.C. Ibe, and K.A. Fasure, *BMC Complement. Altern. Med.* 5, 1 (2005).
3. J.H. Doughari, *Trop. J. Pharm. Res.* 5, 597 (2006).
4. J. Calixto, *Braz. J. Med. Biol. Res.* 33, 179 (2000).
5. C.W. Fennel, K.L. Lindsey, L.J. McGaw, O.M. Grace, and J. VanStaden, *J. Ethnopathol.* 94, 205 (2004).
6. R.A. Street, W.A. Stirk, and J. VanStaden, *J. Ethnopharmacol.* 119, 205 (2008).
7. A. Nostro, M.P. Germano, V.D. Angelo, A. Marino, and M.A. Cannatelli, *Lett. Appl. Microbiol.* 30, 379 (2000).
8. A. Samie, C.L. Obi, P.O. Bessong, and L. Namrita, *Afr. J. Biotechnol.* 4, 1443 (2005).
9. D.A. Akinpelu and T.M. Onakoya, *Afr. J. Biotechnol.* 5, 1078 (2006).
10. J. Parekh and S. Chanda, *Afr. J. Biotechnol.* 6, 766 (2007).
11. M.I. Okeke, C.U. Iroegbu, C.O. Jideofor, A. Okoli, and C.O. Esimone, *J. Herb spices Med. Plants* 8, 39 (2001).
12. U.J.J. Ijah and F.O. Oyebanji, *Glob. J. Pure Appl. Sci.* 9, 193 (2003).
13. A.O. Ogundare, F.C. Adetuyi, and F.A. Akinyosoye, *Afr. J. Biotechnol.* 5, 1663 (2006).
14. H. White, *Medicine and Wart Removal, Hemorrhoids Treatment and Herpes Prevention Without Drugs* (McGraw-Hill, New York, 2006). pp. 102–105.
15. R.N. Okigbo and M. Igwe, *Acta Microbiol. Immunol. Hung.* 54, 353 (2007).
16. A.M. Oloyede, A.O. Aduramigba-Modupe, and I.K. Efem, *Nat. Sci.* 10, 43 (2012).
17. D.K. Olukoya, N. Idika, and T. Odugbemi, *J. Ethnopharmacol.* 39, 69 (1993).
18. R.A. Onyeagba, O.C. Ugbogu, C.U. Okeke, and O. Iroakasi, *Afr. J. Biotechnol.* 3, 552 (2004).
19. S.A. Sakir and W.M. Al-Amoudi, *J. Appl. Pharm. Sci* 02, 22 (2012).
20. F.O. Omoya and F.C. Akharaiyi, *Int. Res. J. Pharm.* 2, 127 (2012).
21. Z.M. Al-Amin, *Br. J. Nutr.* 96, 660 (2006).
22. A.T. Afshari, *Food Chem.* 101, 148 (2007).
23. A. Sebiomo, A.D. Awofodu, A.O. Awosanya, F.E. Awotona, and A.J. Ajayi, *J. Microbiol. Antimicrob.* 3, 18 (2011).
24. P.N. Ravindran and K. Nirmal Babu, *Botany and Crop Improvement of Ginger—The Genus Zingiber* (CRC Press, 2005).
25. A. Cakir, S. Kordali, H. Zengin, S. Izumi, and T. Hirata, *Flavour Frag. J.* 19, 62 (2004).
26. E.E. Elgorashi, J.L.S. Taylor, A. Maes, M. deKimpe, J. VanStaden, and L. Verschaeve, *S. Afr. J. Bot.* 68, 408 (2002).
27. N.S. Mashhadi, R. Ghiasvand, G. Askari, M. Hariri, L. Darvishi, and M. Rezamofid, *Int. J. Prev. Med.* 4, S36 (2013).
28. C.O. Eleazu, K.C. Eleazu, E. Awa, and S.C. Chukwuma, *Eur. J. Biotechnol. Pharm. Res.* 3, 42 (2012).
29. S. Nair, A. Sasidharan, V.V.V.D. Rani, D. Menon, S. Nair, K. Mazoor, and S. Raina, *J. Mater. Sci. Mater. Med.* 20, 235 (2009).
30. N. Padmavati and R. Vijayaraghavan, *Sci. Technol. Adv. Mater.* 9, 1468 (2008).
31. R. Hardman, *Environ. Health Perspect.* 114, 165 (2006).
32. S.P. Malu, G.O. Obochi, E.N. Tawo, and B.E. Nyong, *Glob. J. Pure Appl. Sci.* 15, 365 (2009).
33. G.E. Treese and W.C. Evans, *Pharmacognosy* (London: Bailliere Tridall, 1996), pp. 89–122.
34. B.A. Forbes, D.F. Sahm, A.S. Weissfeld, and E.A. Trevino, *Bailey Scott's diagnostic microbiology*, ed. E.J. Baron, L.R. Peterson, and S.M. Finegold (St Louis: Mosby Co., 1990), pp. 171–194.
35. S. Omar, B. Lemonnier, N. Jones, C. Ficker, M.L. Smith, C. Neema, G.H.W. Towers, K. Goel, and J.T. Amason, *J. Ethnopharmacol.* 73, 161 (2000).
36. M.N. Igwo-Ezikpe, N.O.A. Imaga, H.A. Ogbunugafor, A.A. Osuntoki, S. Adeleye, and A.O. Ipadeola, *The Bioscientist* 1, 73 (2013).
37. M. Durandurdu, *J. Phys. Chem. Solids* 70, 645 (2009).
38. H.M.M. Ibrahim, *J. Radiat. Res. Appl. Sci.* 8, 265 (2015).
39. G.D. Lutterodt, A. Ismail, R.H. Basheer, and H.M. Baharudin, *Malays. J. Med. Sci.* 6, 17 (1999).
40. M.C. Marjorie, *Clin. Microbiol. Rev.* 12, 564 (1999).
41. S. Gur, D. Turgut-Balik, and N. Gur, *World J. Agric. Sci.* 2, 439 (2006).
42. G. Singh and S.V. Bhat, *J. Appl. Phy.* 111, 123913 (2012).
43. C. Malarkodi, S. Rajeshkumar, K. Paulkumar, G. GnanaJobitha, M. Vanaja, and G. Annadurai, *Adv. Nanores.* 1, 83 (2013).
44. D. Kim, S. Jeong, and J. Moon, *Nanotechnology* 17, 4019 (2006).
45. M. Vanaja, G. Gnanajobitha, K. Paulkumar, S. Rajeshkumar, C. Malarkodi, and G. Annadurai, *J. Nanostruct. Chem.* 3, 1 (2013).