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PHYTOCHEMICAL SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES SYNTHESIZED BY AQUEOUS RHIZOMIC EXTRACT OF ALPINIA GALANGA AND EXPLORING ITS ANTIMICROBIAL POTENTIAL

Biotechnology

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ABSTRACT

The field of nanotechnology has recently witnessed spectacular advances in the methods of nanomaterial fabrication and utilizing their exotic physicochemical and optoelectronic properties. Nanoparticles exhibited "Brownian movement" in suspensions and the particle size distribution can be determined using scattering analysis. A. galanga derived AgNPs confirmed the particles size distribution about 169.9 nm. The zeta potential of the particles, however, strongly depends on the pH and the electrolyte concentration of the dispersion. Supporting the above statement, AgNPs dispersed in physiological saline was highly stable with a zeta potential value of −12.6 mV. Antimicrobial activity of biological compound was studied by agar well diffusion method, killing curve assay, DNA fragmentation analysis, SDS-PAGE and in silico interaction studies.

KEYWORDS

Alpinia galanga, aqueous extract, Rhizome, Antimicrobial.

INTRODUCTION

Infectious diseases are the major public health concerns worldwide and accounts for considerably high cases of illness. Studies report that *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, *Shigella* and *Staphylococcus aureus* are considered to be the most frequent pathogens [1]. According to the WHO more than 80 % of the world's population relies on traditional herbal medicine for their primary health care [2]. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants [3]. In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. Nanotechnology is one of the exciting fields with many applications in the modern medicine [4]. They are able to absorb or encapsulate a drug or a chemical thus protecting it against chemical and enzymatic degradation [5].

MATERIALS AND METHODS

Preparation of *A. galanga* Rhizome Extract:

The plant material (rhizome of *A. galanga*) was collected from the local market, Tiruchirapalli and was authenticated. The rhizomes were washed well on the running water to remove any possible impurities. It was shade dried for a week completely to remove the moisture, chopped into small pieces and ground well in mixer into a fine powder.

Preparation of Aqueous Extract:

5 g of prepared rhizome powder was weighed and mixed in 100 ml of deionized water to make it up to 5% concentration of aqueous extract was prepared. Then mixture was boiled in water bath for 10 minutes at 60 °C. After that mixture is cooled at room temperature, the mixture was filtered by using Whitman filter paper. The filtered aqueous solution/extract was used for the further study.

Biosynthesis of Silver Nanoparticles:

The procedure followed for the preparation of the nanoparticles was the protocol used by (Song and Kim 2009) [6]. 5 ml of 5% prepared aqueous solution was added to 95 ml of aqueous silver nitrate (Qualigens – 99.8%) solution. The temperature effects on the synthesis rate and particle size/shape of the prepared silver nanoparticles were studied by reactions that take place in the water bath at 90 °C. The heated mixture of silver nanoparticle solution was then purified by repeated centrifugation at 10,000 rpm for 10 minutes. The supernatant was discarded and pellet was dispersed in deionised water. The activity of centrifugation and redispersion in sterile distilled water was done repeatedly for three times to make sure that there is a better separation of free entities from the metal nanoparticles.

CHARACTERIZATION OF SYNTHESIZED SILVER NANOPARTICLES:

UV- Visible Spectroscopy Analysis:

The preliminary characterization of the silver nanoparticles was carried out using UV-visible spectroscopy. Noble metals, such as silver (Ag) and gold (Au) nanoparticles exhibit unique and tuneable optical properties on account of their Surface Plasmon Resonance (SPR), depending upon shape, size and size distribution of the nanoparticles [7]. The reduction of silver ions was monitored by measuring the UV–visible spectra of the solutions after diluting a small aliquot (0.2 mL) of the aqueous component. The nanoparticles solution was diluted to 20 times with deionized water to avoid errors due to high optical density of the solution. Deionised water was used as a blank.

FT-IR (Fourier Transform Infra-Red Spectroscopy) Analysis:

FT-IR is most useful for identifying types of chemicals that are either organic or inorganic. It can be utilized to quantitative various components of an unknown mixture. It can be applied to the analysis of solids or liquids. The purified liquid of silver nanoparticles in the study was subjected to Fourier Transform Infra-Red spectroscopy analysis (FT-IR) for the analysis of functional groups encapsulated on the synthesized nanoparticles.

These measurements were carried out on a Spectrum RX 1 model instrument in the diffuse reflectance mode at a resolution of 4 cm-1 in potassium bromide (KBr) pellets. For comparison, a drop of 5% *A. galanga* was mixed with KBr powder and pelletized after drying properly. The pellets were later subjected to FTIR spectroscopy measurement and the spectra were recorded in the 4000- 400 nm-1 range.

Transmission Electron Microscopy (TEM) Analysis:

Silver nanoparticle Samples for Transmission Electron Microscopy (TEM) analysis were prepared by drop-coating Ag nanoparticle solutions onto carbon-coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min, following which the extra solution was removed using a blotting paper and the grid was allowed to dry, prior to measurement. TEM measurements were performed on a Tecnai 10 Model instrument operated at an accelerating voltage at 80 kV to determine the shape and size of the silver nanoparticles.

Scanning Electron Microscope:

25µl of sample was sputter coated on gold stub and the images of nanoparticles were studied using Scanning Electron Microscope (SEM).

ANTIMICROBIAL ACTIVITY

Test-Microorganisms: Gram negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, Gram positive *Staphylococcus aureus* and *Bacillus subtilis* and a yeast pathogen *Candida albicans* were used for *in vitro* antimicrobial activity.

In Vitro Antimicrobial Activity:

The antimicrobial activity was determined by agar well diffusion [8]. 25 ml of molten Mueller Hinton Agar was poured into a Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown 100 µl of above said pathogenic microorganisms

cultures were transferred onto plate and made culture lawn by using sterile L-rod spreader. After 5 min 5 wells were made in the plates with the help of a cork-borer (5 mm). The test samples were dissolved in DMSO at various concentrations (i.e. 50, 100, 150 and 200µg/mL). The wells were loaded with various concentrations of test samples and commercial drug Streptomycin served as control. The plates were incubated at 37°C for 24 h. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition around the well.

Time to Kill Assay:
The bacteriostatic and minimum bactericidal concentration (MBC) effects of the sample were determined by a time-kill assay (CLSI, 1997). *S. aureus* culture was grown overnight at 37°C in MHB with aeration and then diluted into fresh MHB with aeration until the cultures reached ~10^6 CFU/mL.

At this point, 2 mL aliquots of culture were removed, and sample was added at one, two, four and eight times the MIC, respectively, as well as the cultures was further incubated at 37°C with aeration. Samples of 100 µL each was withdrawn from the cultures at 0, 1, 4, 8 and 24 h and was serially diluted in cold 0.9% sodium chloride, and plated in duplicate on MHA plates. The plates were incubated for 24 h at 37°C, and the colonies on each plate were counted. The CFU per mL was calculated for each time interval at every purified molecule's concentration. The percentage of bacteria killed was determined by the following equation: 100 × (log10 CFU per milliliter killed at end of incubation period with purified molecule)/ (log10 CFU per milliliter at end of incubation period without purified molecule). In this study, killing was defined as ≥99.9% of the final inoculum as 100% or total killing.

DNA Fragmentation Assay:
For DNA fragmentation detection in *S. aureus* treated with sample concentration of 500 µg/mL in a broth culture for 24 h and then were centrifuged at 3000 rpm for 5 min. Cells were washed twice with phosphate buffered saline, pelleted, and incubated in 200 µL of lysis buffer containing 10 mM Tris–HCl and 10 mM EDTA (pH 8.0), 5% SDS, and 1 mg/mL proteinase K) for 1 h at 55°C and treated with 0.5 mg/mL RNase. DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1 v/v) and precipitated with 100 µL ammonium acetate of 7.5 M and 3 volumes of absolute ethanol. *S. aureus* without any drug treated DNA sample served as control. DNA fragmentation was analyzed by agarose gel electrophoresis for 1.5 h at 50 V on a 1% agarose gel containing ethidium bromide, then observed and photographed under UV light.

Protein Synthesis Inhibition Assay:
The detection of protein synthesis inhibition was examined in the *S. aureus* treated with sample concentration of 500 µg/mL in a broth culture for 24 h and then were centrifuged at 3000 rpm for 5 min. Cells were homogenized in cold 50 mM phosphate buffer pH (7.4) and centrifuged at 10,000 rpm for 15 min. The resultant supernatant was collected and 85% ammonium sulphate was added and kept for overnight at 4°C. Salt precipitant was obtained by centrifugation at 10,000 rpm for 15 min at 4°C. The precipitant was dialyzed on 50 mM phosphate buffer overnight. Dialyzed sample was analyzed in 10% SDS-PAGE.

GC-MS/MS Analysis:
GC-MS analysis was performed using an ITQ900 Thermo Fisher Instrument, Holy cross College, Trichy. The machine was run with fused silica capillary column. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 1µl was employed. Injector temperature was set at 250°C and the ion-source temperature as 280°C.

The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST).

***IN SILICO* STUDIES**
Protein Data Bank (PDB):
PDB has been operated by the Worldwide Protein Data Bank. Collaboration of organizations that act as deposition, data processing and distribution centers for PDB data. The three dimensional structure of Autolysin (PDB ID: 2B0P) was obtained from protein data bank.

Receptor Preparation:
The ligands and crystallographic water molecules were removed from the protein, and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of presentation, the protein was subjected to energy minimization using the CHARMM force field.

Active Site Analysis:
The active site is usually a big pocket or cleft surrounded by amino acid and other side chains at the surface of the enzyme, these residues responsible for the substrate specificity and catalytic residues which often act as proton donors or acceptors. Identification and characterization of binding sites is key process of drug design. Active site residues of Autolysin were predicted using the option in Discovery studio.

Ligand Preparation:
The three dimensional structures of phytocompounds were drawn by Chemsketch software and saved in .mdl format to download in Discovery studio 2.1. Hydrogen bonds were added and the energy was minimized using CHARMM force field. Lipinski's properties like molecular weight, log P and number of Hydrogen-bond donors and acceptors for the active principles were noted.

Docking – Discovery Studio:
The docking analysis was carried out by using discovery studio 2.1**.** It makes easier to examine the properties of large and small molecules. DS unites powerful algorithm for flexible docking, protein ionization and ligand scoring. The receptor and ligand prepared was docked using this software to find the receptor-ligand interaction. The presence of hydrogen bond and other interactions were found for the best pose of interaction with high absolute energy and Libdock score.

RESULTS
Change in color of the solution (white to brown) indicates that the rhizome extract of *Alpinia galanga* has the potential to reduce silver nitrate ions to silver nanoparticles. The color change is due to the excitation of Surface Plasmon vibration in nanoparticles [9]. Dark brown color was noticed when the solution was incubated at 90ºC and hence 90ºC was used for further analysis. The reduction of silver ions was monitored by measuring the UV-Visible Spectrum of the reaction medium after diluting in double distilled water. Biologically synthesized nanoparticles at various temperatures indicated an absorbance peak at 30ºC - 379 nm, 60ºC – 417 nm and at 90ºC – 437 nm respectively.

Fig 1: Color changes of Silver nanoparticles synthesized from *A. galanga* rhizome

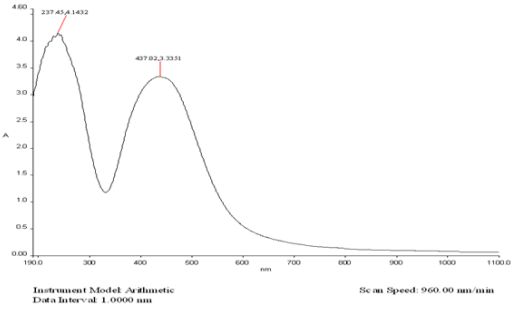


Fig 2: UV-Vis spectroscopy reading of synthesized SNPs

The FTIR spectrum of silver nanoparticles synthesized using *Alpinia galanga* at various temperatures shows peaks at 3433.78 cm^{-1}, 2369.89 cm^{-1}, 2080.65 cm^{-1}, 1637.31 cm^{-1} and 674.85 cm^{-1}. The phenols, alkenes, amines, alkynes and alkyl halides functional groups was found significantly with biosynthesized silver nanoparticles from *A. galanga* was confirmed.

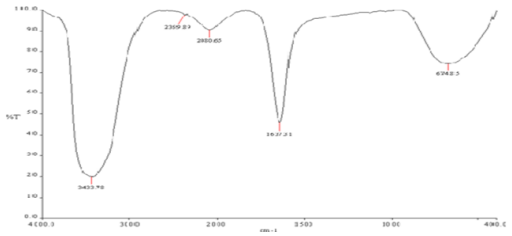


Fig 3: FT-IR reading of synthesized SNP

The aqueous extract treated with silver nitrate solution expressed the synthesis of silver nanoparticles and the size of the particles was confirmed by the TEM. The images were recorded from drop coated film of the silver nanoparticles, which were synthesized by treating silver nitrate solution with aqueous extract of *A. galanga*. The images showed broad size distribution of particle size ranges about 26.55nm around 500 nm.

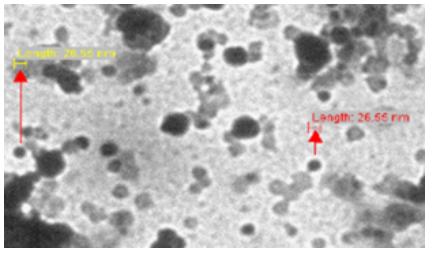


Fig 4: TEM image of biologically synthesized SNPs using *Alpinia galanga*

According to the results observed in the UV-Vis spectroscopy, the temperature at which the maximum synthesis has been achieved was taken for the structural characterization using SEM. The analysis was done for understanding the topology and size of the AgNPs. Most of the particles were rods and cubes.

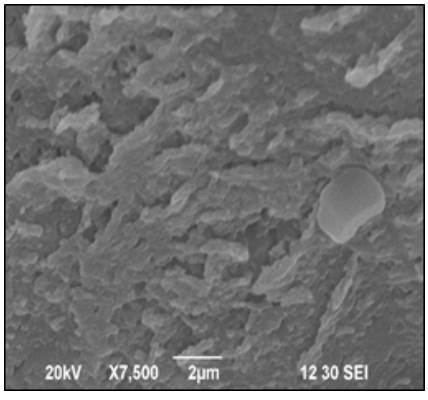


Fig 5: SEM image of biologically synthesized SNPs using *Alpinia galanga*

Zeta potential of the synthesized nanoparticles has been investigated. Zeta potential values reveal details about the surface charge and stability of the synthesized metal nanoparticles. Zeta potential value is – 12.6, which show that surface charge is negative and this reveals that the synthesized nanoparticle is stable.

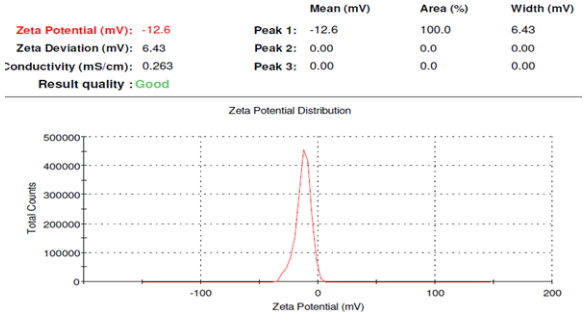


Fig 6: Zeta potential of the synthesized silver nanoparticle

National Nanotechnology Initiative (NNI) defines nanotechnology in dimensions of roughly 1 to 100 nanometers (nm) but in broader range it can be extended up to 1000 nm. Particles that fall within this range appear to be optimal for achieving a number of important tasks. The particle size of the synthesized nanoparticle is found to be 169.9.

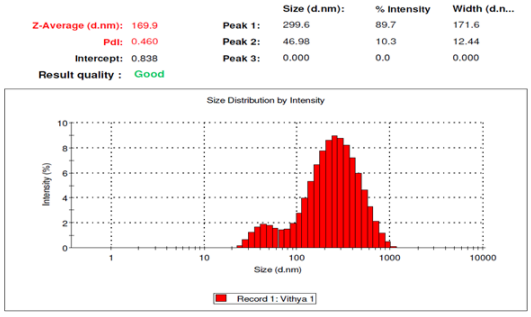


Fig 7: Particle size of the synthesized silver nanoparticle

ANTIMICROBIALACTIVITY

The antimicrobial activity profile of test sample is given in Table 1. Among the test bacterial pathogen, *S. aureus* is most susceptible bacteria and it was taken for the further analysis. 50g/mL test sample showed activity against *S. aureus*, which is comparatively high in other organisms.

Table 1: Antimicrobial activity of given test sample

Organism Name	Antimicrobial activity in zone of inhibition (ZOI) mm* Sample Concentration µg/mL)			
	50	100	150	200
Staphylococcus aureus	6±1	9±0.6	14±0.6	16±1.5
Bacillus subtilis	8±0.6	9±1	11±1.5	12±1
Pseudomonas aeruginosa	0±0	6±1.5	8±1	9±0.6
Klebsiella pneumoniae	7±1	8±1	9±0.6	12±1
Candida albicans	7±0.6	9±0.6	10±1	12±1.5

*Values are means of triplicate with standard deviations

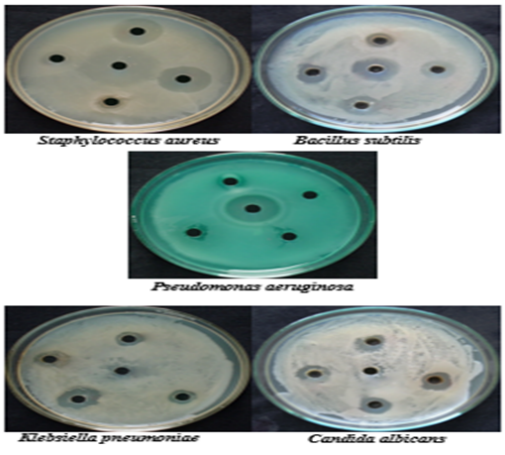


Fig 8: Antimicrobial activity of test sample

Killing Curve Assay:

The bacteriostatic and bactericidal activity of the test sample was determined by a time-kill assay. A time-kill study of S. aureus showed time- and concentration-dependent killing with a 99% decrease in bacterial viability within 8 h at test sample concentration four times greater than the MIC and complete killing within 4 h at concentration eight times greater than the MIC. Bactericidal activity was observed at 24, 8, and 4 h at 1× and 2× 4× and 8× the MIC, respectively.

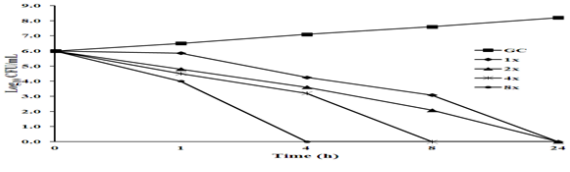
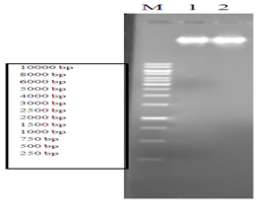


Fig 9: Time-kill assay of the test sample

DNA Fragmentation Assay:

DNA cleavation assay revealed that there was no change in the genomic DNA of test sample treated *S. aureus*.

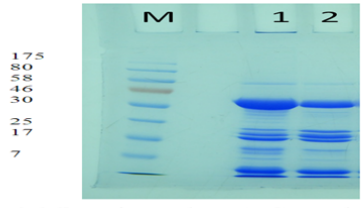


Lanes M: DNA ladder; 1: Control; 2: Treated

Fig 10: DNA cleavation assay of test sample against *S. aureus*

Protein Synthesis Inhibition Assay:

In the point of protein inhibition assay the number of protein band is less number in test sample treated *S. aureus* than the untreated control bacterial cell protein. In addition the protein concentration was very faint in treated cell protein.



Lanes M: Protein marker; 1: Control; 2: Treated

Fig 11: SDS PAGE analysis of *S. aureus* treated with test sample

GC-MS/MS Analysis:

Various phyto-chemical compounds were observed in GC-MS/MS analysis. Highest peak was observed as 25.67 and the compound held at the highest peak was observed to be 6-(3, 5-Dimethyl-furan-2yl)-6-methyl-hept-3-en-2-0ne.

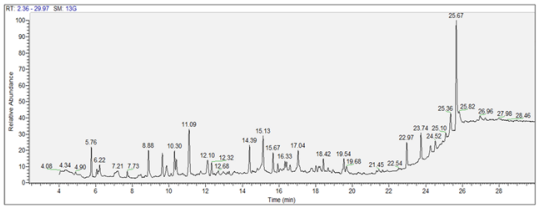


Fig 12: GC-MS/MS analysis of *A. galanga*

***IN SILICO* INTERACTION STUDIES:**

The docking analysis performed to identify the stable interaction between the receptor and the ligand is represented in Fig: 15. The interaction between DMF present in the *A. galanga* extract with the cell division enhancing protein Autolysin present in *S. aureus* resulted in a stable interaction between them. The interaction was between the Asn 303 and Tyr 211 amino acid residues of the receptor which are the active sites of the receptor with the ligand and the bond length was found to be more than 2 indicating the stability of the interaction with high Libdock score and absolute energy.

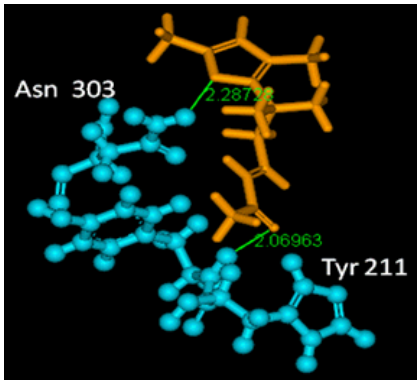


Fig 13: Compound 6-(3, 5-Dimethyl-furan-2yl)-6-methyl-hept-3-en-2-0ne docked with Autolysin receptor

SUMMARY AND CONCLUSION

This study demonstrates antimicrobial activity of *A. galanga* derived AgNPs against Gram negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, Gram positive *Staphylococcus aureus* and *Bacillus subtilis* and a yeast pathogen *Candida albicans*. The maximum peak at 437nm range confirmed the synthesis of silver nanoparticles by their surface plasmon resonance vibration in the aqueous extract. With reference to the FTIR analysis, the phenols, alkenes, amines, alkynes and alkyl halides functional groups was found significantly with biosynthesized silver nanoparticles from *A. galanga* was confirmed. Scanning Electron Microscopy elucidated the structure of polydispersed rods and cubical AgNPs in which the average particle size was confirmed to be 26nm in Transmission Electron Microscopy.

Zeta potential and particle size analysis shows that the synthesized nanoparticles are stable and the size is below 200nm. Among the test bacterial pathogen, *S. aureus* is most susceptible bacteria and it was taken for the further analysis. 50g/mL test sample showed activity against *S. aureus*, which is comparatively high in other organisms. DNA cleavation assay revealed that there was no change in the genomic DNA of test sample treated *S. aureus* but in the point of protein inhibition assay the number of protein band is less number in test sample treated *S. aureus* than the untreated control bacterial cell protein. In addition the protein concentration was very faint in treated cell protein. Thus the present study confirms the ability of the test plant for having the active compounds to synthesize of silver nanoparticles using biomimetic method and exploring antimicrobial activity.

REFERENCES:

1. Abdulmajeed NA (2010). Therapeutic ability of some plant extracts on Aflatoxin BL induced renal and cardiac damage. Arabian journal of chemotherapy. 4(1): 1-10.
2. Adams M; Gmunder F; Hamburger M (2007). Plants traditionally used in age related brain disorders- a survey of ethnobotanical literature. Journal of Ethnopharmacology. 113(3): 363-381.
3. Ahmad Z; Pandey R; Sharma S; Khuller GK (2005). Alginate Nanoparticles as Antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. Indian Journal of Chest Diseases. Allied Sciences. 48:171–6.
4. Albrecht MA; Evan CW; Raston CL (2006). Green chemistry and the health implications of Nanoparticles. Journal. Green Chem. 8:417–32.
5. Al-Yahya MA; Rafatullah S; Mossa JS; Ageel AM; Al- Said MS; Tariq M (1990). Gastric Antisecretory, Antiulcer and Cytoprotective properties of Ethanolic extract of Alpina galanga Wild. in rats. phytother Res 4, 112-114.
6. Berns, J.S. (2003). Infection with Antimicrobial-resistant Microorganism in Dialysis patients. Seminars in Dialysis. 16: 30-37.
7. Borris, R.P. (1996). Natural productions research: perspectives from a major pharmaceutical company, Journal of Ethnopharmacology. 51: 29-38.
8. Boyce, J.M. (1992). Methicillin-resistant Staphylococcus aureus in hospitals and long-term care facilities: Microbiology, Epiderminology and Preventive measures. Infection Control Hospital Epidemiology. 13, pp. 725-737.
9. C. J. Murphy, (2008). Sustainability as an Emerging Design Criterion in Nanoparticle Synthesis and Applications. Journal of Material Chemistry. 18, 2173.
10. Chan EWC; Lim YY; Omar M (2007). Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. Food chemistry. 104(4): 1586-1593.
11. Cox, P.A. (1994). The Ethnobotanical Approach to Drug Discovery: Strengths and limitations. In Prance, G.T. (eds) Ethnobotany and the Search for New Drugs. Wiley, Chichester, U.K. P 25-41.
12. Dhruba J Bharali; Marianne Khalil; Mujgan Gurbuz; Tessa M Simone; Shaker A Mousa (2009). Nanoparticles and Cancer Therapy: A Concise review with emphasis on dendrimers. International Journal of Nanomedicine. (4)1–7.
13. Di pietro, S.; Haritchabalet, K.; Cantoni, G.; Iglesias, L.; Manchi, S.; Temperoni, A.; Labanchi, J.L.; Barbarossa, N.; Garcia, M.T.; Cofre, M. et al., (2004). Surveillance of Food Borne Diseases in the Province of Rio Negro, Argentina, 1993-2001. Medicina (B Arires) 64(2): 120-124.
14. Duran N; Marcarto PD; De Souza GIH; Alves OL; Esposito E (2007). Antibacterial effect of silver Nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of Biomedical Nanotechnology. 3:203–8.
15. Feng QL; Wu J; Chen GQ; Cui FZ; Kim TN; Kim JO (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of Biomedical Materials. 52(4):662–8.
16. Feynman R. (1959). Lecture at the California Institute of Technology. December 29.
17. Ficker CE; Smith ML; Susiarti S, Leamanb DJ; Irawati C; Arnason JT (2003). Inhibition of human pathogenic fungi by members of Zingiberaceae used by Kenyah (Indonesian Borneo). Journal of Ethnopharmacology. 85(5), 289-293.
18. Fox CL; Modak SM (1974). Mechanism of silver sulfadiazine action on burn wound infections. Antimicrobial Agents Chemotherapy. 5(6):582–8.
19. Furno F; Morley KS; Wong B; Sharp BL; Arnold PL; Howdle SM (2004). Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. Journal of Antimicrobial Chemotherapy. 54:1019–24.
20. Gardea-Torresdey JL; Parsons JG; Gomez E; Peralta-Videa J; Troiani HE; Santiago P; Jose-Yacaman M (2002) Formation and Growth of Au Nanoparticles inside live Alfalfa plants.
21. Gardea-Torresdey L; Gomez E; Peralta-Videa JR; Parsons JG; Troiani H; Jose-Yacaman M (2003) Alfalfa sprouts: A natural source for the synthesis of silver Nanoparticles. Langmuir. 19:1357– 1361.
22. Gaulin, C.; Viger, Y.B.; Filklion, L. (2002). An Outbreak of Bacillus cereus implicating a Part-time Banquet Caterer. Canadian Journal of Public Health; Vol 93, ISSU 377; 353-355.
23. Gong P; Li H; He X; Wang K; Hu J; Tan W, et al. (2007). Preparation and Antibacterial activity of Fe3O at Ag Nanoparticles. Nanotechnology 18:604–11.
24. GU H; Ho PL; Tong E; Wang L; Xu B (2003). Presenting Vancomycin on Nanoparticles to Enhance Antimicrobial activities. Nano Letters. 3(9):1261–3.
25. H.S. Naiwa, Ed. Handbook of Nanostructural Materials and Nanotechnology Academic Press.

26. Hall, G.; Kirk, M.D.; Becker, N.; Gregory, J.E.; Unicomb, L.; Millard, G.; Stafford, R.; Lalor, K.; Ozfoodnet (2005). Estimating Food Borne Gastroenteritis, Australia. Emerging Infectious Disease. 11(8)1257-1264.
27. Indrayan AK; Agrawal P; Rathi AK; Shatru ; Agrawal NK; Tyagi DK (2009). Nutritive value of some indigenous plant rhizomes resembling Ginger. Natural Product Radiance. 8(5): 507-513. Interface Science. 275:496-502.
28. Ip M; Lui SL; Poon VKM; Lung I; Burd A (2006). Antimicrobial Activities of Silver Dressings: an In vitro comparison. Journal of Medical Micro biotechnology. 55:59–63.
29. Jain P; Pradeep T (2005). Potential of Silver Nanoparticle-coated Polyurethane foam as an Antibacterial water filter. Journal of Biotechnology Bioengineering. 90(1):59–63.
30. Jaju S; Indurwade N; Sakarkar D; Fuloria N; Ali M (2009). Isolation of galangogalloside from rhizomes of Alpinia galangal. International Journal of Green Pharmacology. 3(2): 144-147.
31. Jaju SB, Indurwade NH, Sakarkar DM, Fuloria NK, Ali MD, Basu SP (2010). Isolation of β-sitosterol diglucosylcaprate from Alpinia galanga. Pharmacognosy Res. 2(4): 264-266.
32. Jun J; Yuan-Yuan D; Shao-hai W; Shao-feng Z; Zhong-yi W (2007). Preparation and characterization of antibacterial silver-containing Nanofibers for wound dressing applications. Journal of US-China Medical Science. 4(2):52–4.
33. Kaur A; Singh R; Dey CS; Sharma SS; Bhutani KK; Singh IP (2010). Antileishmanial phenylpropenoids from Alpinia galanga (Linn) Wild. Indian Journal of Experimental Biology. 48 (3): 314-317.
34. Kirtikar, K. R. & Basu, B.D. (1987). Indian Medicinal Plants. Internet Book Distributors, Dehra Dun. P 2444-2449.
35. Kumar A; Vemula PK; Ajayan PM; John G (2008). Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. Nature Materials. 7(3):236–41.
36. Latha C; Shriram VD; Jahagirdar SS; Dhakephalkar PK; Rojatkar SR (2009). Antiplasmid activity of 1'-acetoxychavicol acetate from Alpinia galanga against multi-drug resistant bacteria. Journal of Ethnopharmacology. 123(3): 522-525.
37. Matsuda H; Pongpiriyadacha Y; Morikawa T; Ochi M; Yoshikawa M (2003). Gastroprotective of phenyl propanoids from the rhizome of Alpinia galanga in rats: structural requirements and mode of action. European Journal of Pharmacology. 471(1), 59-67.
38. Matsuura T; Abe Y; Sato K; Okamoto K; Ueshige M; Akagawa Y (1997). Prolonged Antimicrobial effect of Tissue conditioners containing Silver Zeolite. Journal of Dental. (25):373–7.
39. Mayachiew P; Devahastin S (2008). Antimicrobial and Antioxidant activities of Indian gooseberry and galangal extracts. LWT Food Science Technology. 41 (7): 1153-1159.
40. Min HJ; Nam JW; Yu ES; Hong JH; Seo EK and Hwang ES (2009). Effect of naturally occurring hydroxychavicol acetate on the cytokine production in T helper cells. International journal of Immunopharmacology. 9(4), 448-454.
41. Mulvaney P (1996). Surface plasmon spectroscopy of Nanosized metal particles. Langmuir 12:788–800.
42. Oonmetta-aree J; Suzuki T; Gasaluck P; Eumbek G (2006). Antimicrobial properties and action of galangal (Alpinia galanga Linn.) on Staphylococcus aureus. LWT Food Science Technology. 39(10): 1214-1220.
43. P.C. Pande; Lalit Tiwari; H.C. Pande; Bishen Singh; Mahendra Pal Singh (2006). Folk Medicine and Aromatic Plants of Uttaranchal. Indian Journal of Traditional Knowledge. PC 6(3) pp. 444-458.
44. Pal S; Tak YK; Song JM (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Journal of Applied and Environmental Microbiology. 27(6):1712–20.
45. Pooter D; Omar HL; Coolsaet MN; Schamp BA (1985). The essential oil of greater galanga (Alpinia galanga) from Malaysia. Phytochemistry. 24, 93-96.
46. Prado, V.; Solari, V.; Alvarez, I.M.; Arellano, C.; Vidal, R.; Carreno, M.; Mamani, N.; Fuentes, D.; Munoz, V. (2002). Epidemiological Situation of Food Borne Diseases in Santiago, Chile in 1999-2000. Revista Medica de Chile 130(5): 495-501.
47. Raimondi F; Scherer GG; Kotz R; Wokaun A (2005). Nanoparticles in energy technology: Examples from electrochemistry and catalysis. Angew. Chem., Int. Ed. 4:2190–209.
48. Rao K; Ch B; Narasu LM; Giri A (2010). Antibacterial activity of Alpinia galanga (L) Wild Crud extracts. Applied Biochemistry and Biotechnology. 162(3): 871-884.
49. Retchkiman-Schabes PS; Canizal G; Becerra-Herrera R; Zorrilla C; Liu HB; Ascencio JA (2006). Biosynthesis and characterization of Ti/Ni bimetallic Nanoparticles. Journal Optical Materials. 29:95–9.
50. Richard JW; Spencer BA; McCoy LF; Carina E; Washington J; Edgar P, et al (2002). Acticoat versus silverlon: the truth. Journal of Burns Surgical Wound Care. 1:11–20.
51. Sawangjaroen N; Subhadhirasakul ES; Phongpaichit ES; Siripanth C; Jamjaroen EK; Sawangjaroen EK (2005). The in vitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand. Parasitol Res. 95, 17-21.
52. Shankar SS; Rai A; Ahmad A; Sastry M (2004). Rapid Synthesis of Au, Ag and Bimetallic Au core Ag Shell Nanoparticles using Neem (Azardirachta indica) Leaf Broth. Journal of Colloid and interface science. Vol-275, P 496–502.
53. Sivarajan, V. V. & Balachandran, I. (1994). Ayurvedic drugs and their Plant Sources. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 570p.
54. Sondi I; Salopek-Sondi B (2007). Silver Nanoparticles as Antimicrobial agent: a case study on E. coli as a model for gram-negative bacteria. Journal of Colloid Interface. 275:177–82.
55. Song JY; Kim BS (2009). Rapid Biological Synthesis of Silver Nanoparticles using Plant Leaf Extracts. Bioprocess Biosyst Eng. 32:79-84.
56. Tachakittirungrod S; Chowwanapoonpohn S (2007). Comparision of Antioxidant and Antimicrobial activities of Essential oils from Hyptis suaveolens and Alpinia galanga growing in Northern Thailand. CMU Journal of Nature Science. 6, 31-42
57. Tammy P. Chou; Qifeng Zhang; Glen E. Fryxell; Guozhong Cao (2007). Hierarchically Structured ZnO Film for Dye-Sensitized Solar Cells with Enhanced Energy Conversion Efficiency. Advanced Materials. (19)2588–2592.
58. Taniguchi N. (1974). On the Basic Concept of Nano-Technology. Proc. Intl. Conf. Prod. Eng. Tokyo, Part II. Japan Society of Precision Engineering.
59. Tewtrakul S; Subhadhirasakul S; Kummee S (2003). HIV-1 Protease inhibitor effects of medicinal plants used as self medication by AIDS patients. Journal of Science and Technology. 25(2), 239-243.
60. Thakur R S; Puri HS; Husain A (1989). Major Medicinal Plants of India, CIMAP, Lucknow, India.pp.50-52. Bisht G. S.; Awasthi A.K. and Dhole T.N. (2006). Antimicrobial activity of Hedychium spicatum. Fitoterapia, 77:240-242.
61. Tian J; Wong KKY; Ho CM; Lok CN; Yu WY; Che CM; Chiu JF; Tam PKH (2006). Topical Delivery of Silver Nanoparticles Promotes Wound Healing. Journal of Medical Chemistry. 00:171–80.
62. Turker A; Usta C (2002). Biological activity of some medicinal plants sold in Turkish Health-food stores. Biodiversity Ecosystem. 34(19), 105-113.
63. Vinutha B; Prashanth D; Salma K; Sreeja SL; Pratiti D; Padmaja R; Radhika S; Amit A; Venkateshwarlu K; Deepak M (2007). Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. Journal of Ethnopharmacology. 109(2): 359-363.
64. Voravuthikunchai, S.P.; Popaya, W.; Supavita, T. (2004). Antibacterial Activity of Crude Extracts of Medicinal Plants used In Thailand against Pathogenic Bacteria. Ethnopharmacologia 33, 60-65.
65. W. Jahn, (1999) Green Synthesis of silver Nanoparticles using Argemone Mexicana Leaf Extract and Evaluation of their Antimicrobial activities. Journal of Structrual Biology. 127, 106.
66. Xia L; Lenaghan SC; Zhang M; Zhang Z; Li Q (2010). Naturally Occurring Nanoparticles from English Ivy: An Alternative to Metal based Nanoparticles for UV Protection. Journal of Nanobiotechnology. 8:12.
67. Y Tijani, M. O.; Uguru, O. A.; Salawu. Anti-Malarial Activity of Ethanolic Stem Bark Extract of Faidherbia Albida (Del) a. Chev (Mimosoidae) in mice. African Journal of Biotechnology. 2008; 7(6): 696-700.
68. Yoh, M.; Frimpong, E.K.; Voravuthikunchai, S.P.; Honda, T. (1999). Effect of Sub Inhibitory Concentrations of Antimicrobial Agents (Quinolones and Macrolide) On the Production and Release of Vero Toxin by Enterohaemorrhagic Escherichia coli O157:H7. Canadian Journal of Public Health Association. 45, 732-739.
69. Zhu XL; Yang MH; Luo JG; Huang XF; Kong LY (2009). A New Phenylpropanoid from Alpinia galanga. Journal of Chinese Integrative Medicine. 7(1): 19-20.