Green tea leaves mediated ZnO nanoparticles and its antimicrobial activity

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Abstract: Plant-mediated synthesis of ZnO Nanoparticles (NPs) have multiple advantages over conventional synthetic methods like easy, inexpensive, eco-friendly, nontoxic by-products and no critical conditions of temperature and pressure required. In this study, 9.1 g ZnO NPs were synthesized from 230 mL of 0.2 M Zinc acetate dihydrate and 100 mL of green tea leaves extract at room temperature (25°C). The leaves extract was prepared by heating 10gm dried leaves in 100 mL of deionized distilled water at 80°C for 2 h. The synthesized ZnO NPs were dried at 40°C for 24 h and calcined at 100°C for 1 h. The agar well diffusion method was used to evaluate ZnO NPs for antimicrobial activity of selected pathogenic strains. A clear zone of inhibition was measured; 40.05 mm ± 0.137 for Staphylococcus aureus, 36.15 mm ± 0.304 for Escherichia coli and 40.10 mm ± 0.050 for Aspergillus niger that were comparably better results than standard antibiotic Gentamycin that showed 25 and 26 mm zone of inhibition for Staphylococcus aureus and Escherichia coli respectively. The minimum inhibitory concentration scored as 9.765µg ± 0.00, 9.531µg ± 0.00 and 5000µg ± 0.00 for Staphylococcus aureus, Escherichia coli and Aspergillus niger respectively, was documented low concentration than reported so far concentrations of green tea ZnO NPs.

Subjects: Bioscience; Food Science & Technology; Physical Sciences; Medicine, Dentistry, Nursing & Allied Health

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PUBLIC INTEREST STATEMENT
Since plant extracts having bioactive constituents are commonly used in pharmaceutical formulations due to their medicinal applications. These bioactive constituents can be involved in reducing and capping of metal ions for the synthesis of nanoparticles (NPs). A few reported works is available on the biosynthesis of some metals NPs like MgO, TiO2, CuO, FeO2, Al2O3 and ZnO. From all these, ZnO NPs has got great attention in recent years with enormous applications as these are easy to synthesize, cheap and safe method. Plant mediated NPs free from toxins can have vast scope in the field of biomedical science, food and cosmetics industries. These NPs are most promising as they show good antibacterial and antifungal properties due to their large surface area to volume ratio, became a current interest in the researches due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains.

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Keywords: green tea; leaves; mediated; nanoparticles; antimicrobial

1. Introduction
Nanotechnology is emerging as multidisciplinary field of science in which a wide range of metal nano nanoparticles (NPs) have been synthesized. The produced NPs have unique size with more surface area to volume ratio that promoted their reactivity with the surrounding molecules (Gunalana, Sivaraja, & Rajendran, 2012). Therefore conventionally both physical and chemical methods are used to synthesize them, however these methods have various demerits including expensive, toxic by-products, critical conditions of temperature and pressure and long-time of reactions etc (Geraldes et al., 2016). Whereas green synthesis of NPs; involve nontoxic, cheap and broadly available plant sources that are environment friendly (Salem, Albanna, & Awwad, 2016). Those medicinal plants were preferably used in the synthesis of NPs that already documented for biomedical properties and having immense range of natural products (Agarwal, Kumar, & RajeshKumar, 2017). These bioactive phytochemicals are reacted to reduce metals into metal oxide and showing good stability in the formation of NPs (Mishra & Sharma, 2015). Noble metals like gold (Au) and silver (Ag) have been extensively used in biosynthesis NPs and medically evaluated. However very few reported work was available on inorganic metals such as Ti, Mg, Fe, Zn, S and Al. Among these metals; ZnO has got excellent position due its wide applications in various fields of science (Dhanemozhi, Rajeswari, & Sathyajothi, 2017).

ZnO NPs were reported to have better UV protection and enhanced opaqueness than TiO₂ NPs that was previously used for UV protections (Sundrarajan, Ambika, & Bharathi, 2015). These NPs showed elevated catalytic, photochemical and antimicrobial properties (Awwad, Albiss, Ahmad, 2014). These NPs were documented to rupture the lipid bilayers of bacterium and fungal cell wall and revealed significant antibacterial and antifungal activity (Senthilkumar & Sivakumar, 2014). Beside this; ZnO NPs were also evaluated to have good antioxidant, anti-diabetic and anticancer property (Pattanayak & Nayak, 2013). Thus, these NPs depicted tremendous applications in biomedicines and microelectronics (Hasan, Das, Khan, Hasan, & Rahman, 2009). Production of ZnO NPs is still infancy stage; therefore there is a gigantic scope of this work to synthesize and evaluate its antimicrobial activity.

In this study, dried Green tea leaves were used to synthesize ZnO NPs. It is scientifically known as “Camellia Sinensis” famous for its Phenolic contents and high antioxidant activity (Saravanakkumar, Sivarajani, Umamaheswari, Pandiarajan, & Ravikumar, 2016). This plant belongs to family Theaceae and is rich in bioactive phytochemicals that refers it as anti-septic, anticancer and antimicrobial agent for valuable medical drugs (Rani, Nagpal, Gullaiya, Madan, & Agrawal, 2014). Consequently, these properties are enhanced in the form of ZnO NPs; where these bioactive components are locked while reducing and capping of NPs (Malapermal, Mbatha, Gengan, & Anand, 2015). During the present work ZnO NPs were synthesized by using leave extract of Camellia sinensis (C. sinensis) and its antibacterial and antifungal activity was evaluated. Further produced ZnO NPs were also characterized by UV–visible, FTIR, XRD and SEM.

2. Materials and methods
The plant material was procured from the local nursery near Gulberg and identified by Dr. Zaheer Uddin Khan, Distinguished Professor, Botany department, Govt. College University, Lahore, Pakistan. The fresh leaves were separated and dried under shade for 5 days. The dried leaves were grinded into power and stored in air tight jar for further work.

2.1. Synthesis of ZnO NPs
Leaf extract was prepared by following Senthilkumar and Sivakumar (2014) with some modifications as; 10 g of dried leaves were heated in 100 mL deionized water at 80°C with continuous stirring for 2 h. It was cooled at room temperature (25°C) and filtered by using whatman filter paper No. 40. Then clear extract was obtained by centrifugation at 4000 rpm for 10 min. Zinc acetate dihydrate solution (0.2 M) was freshly prepared and 230 mL was added to 100 mL of leave extract. With the
instant formation of pale yellow ZnO NPs, the reacted solution was dried at 40°C for 24 h and brown
dried crystals were attained. These crystals were further calcined for 1 h at 100°C, cooled, weighed
and stored in brown bottles for future investigations. Freshly prepared 100 mL leave extract was also
dried at 40°C for 24 h for further analysis.

2.2. Antimicrobial studies
The synthesized ZnO NPs were evaluated for antimicrobial activity; well diffusion method (Hasan
et al., 2009) was applied to screen famous pathogenic microbes. For antibacterial assay, gram
positive bacteria *Staphylococcus aureus* (S. aureus) and gram negative bacteria *Escherichia. coli*
(E. coli) were spread uniformly on nutrient agar plates having wells of 4 mm. ZnO NPs (100 mg/mL)
normal solution was introduced in the wells under sterilized conditions and incubated at 37°C for
24 h. The zone of inhibition (mm) around well was measured; same procedure was applied for
pathogenic fungus: *Aspergillus niger* (A. niger) during antifungal assay. All the bacterial and fungal
strains used in this study were obtained from the Department of Microbiology, University Of
Veterinary and Animal Sciences, Lahore. Gentamycin (100 mg/mL) was used as standard antibiotic
against bacterial strains and zone of inhibition (mm) was measured.

2.3. Minimum inhibitory concentration
Minimum inhibitory concentration (MIC) was measured following well diffusion method in repli-
cates (N = 3) for each microbial strain. This standard method for antimicrobial assay (Mishra,
Sasmal, & Shrivastava, 2012) was employed in tube serial dilutions of ZnO NPs (100mg/mL) in
bacterial and fungal growth media. The pathogenic microbes were incubated at 37°C for 24 h and
lowest inhibitory concentration was scored. All the experiments were applied in replicates (N = 3)
and mean value with standard deviation (SD) was calculated by descriptive analysis on SPSS
statistics 17.0.

2.4. Characterization
The presence or absence of important bioactive molecules like alkaloids, flavonoids, phenols,
carbohydrates, protein, terpenes and saponins were investigated by performing reported tests
(Tiwari, Kumar, Kaur, Kaur, & Kaur, 2011). The green tea mediated ZnO NPs were scanned for UV–
visible spectroscopy between 200 and 500 nm to determine absorption maxima. The different
functional groups in crude dried leave extract and ZnO nanoparticles were identified by FTIR
(Agarwal et al., 2017) performed at Centre of Applied Chemistry, PCSIR Laboratories complex,
Lahore, Pakistan. XRD and SEM analysis was achieved at Department of Chemistry, SBASSE Lahore
University of Management Sciences, Lahore, Pakistan.

3. Results and discussion
The green synthetic method of ZnO NPs is a recent approach that is the elucidation of a cheap,
eco-friendly and scale up synthetic method. Medically important plants have such phytochemicals
which act to stabilize and reduce metal oxides for the synthesis of NPs with controlled shape and
size (Rani et al., 2014). Further such famous phytochemicals are involved in the inhibition mechan-
ism of microbial pathogenic growth (Zhang, Ding, Povey, & York, 2008). Therefore, such plant
mediated NPs free from toxins can have vast scope in the field of biomedical science, food and
cosmetics industries, consequently this study now become a foremost area of research. In this
present study, for the green synthesis of ZnO NPs; dried grinded leaves of *C. sinensis* were used to
prepare extract and 230 mL of 0.2 M solution of Zinc acetate dihydrate was poured in 100 mL of
fresh leaf extract. Pale yellow ZnO nanoparticles were instantly appeared that grew larger within
seconds and finally settled down leaving supernatant layer which was also taken for phytochem-
ical investigation. The visual diagram of all the procedure in the form of flow sheet was presented
in Figure 1. Further the produced NPs were dried at 40°C in oven for 24 h and dried brown NPs were
obtained by calcined at 100°C for 1 h. Thus green synthesized ZnO NPs were weighed as 9.1g/
100 mL of leave extract. This is the first time reported concentration of green tea ZnO NPs with
complete scheme of work.
3.1. Antimicrobial activity

The microbicidal activity of synthesized NPs (100mg/mL) was measured against pathogenic strains and found 40.05 mm ± 0.137 zone of inhibition against *S. aureus*, 36.15 mm ± 0.304 for *E. coli* and 40.10 mm ± 0.050 for *A. niger* shown in Figure 2. These results documented better antibacterial activity of produced ZnO NPs than the standard antibiotic; Gentamycin (100 mg/mL) that showed zone of inhibition 25 mm against *S. aureus* and 26 mm for *E. coli*. The biocidal action of ZnO NPs revealed their mechanism that involve the disruption of cell membrane with the action of Zn$^{+2}$ on its surface that ultimately cause the death of microbes (Gunalana et al., 2012). Further standard protocols were followed to measure MIC for the above mentioned strains and observed concentrations for *S. aureus* was 9.765 µg ± 0.00, *E. coli* was 19.531 µg ± 0.00 and *A. niger* was 5000 µg ± 0.00. This minimum concentration of ZnO NPs required for antimicrobial activity as given in Table 1 depicted the cost effectiveness of initially green synthesized ZnO NPs (91 g/100 mL) and its application in antimicrobial activity. Some researchers also studied mode of inhibitory action of ZnO NPs for microbial growth, as Mishra et al. (2012) documented cell damage caused by these NPs with the presence of protein and nucleic acid of nutrient agar, Femi, Prabha, Sudha, Devibala, and Jerald (2011) demonstrated the surface binding of NPs with thiol group of glycoproteins one the cell wall of microbes and decreases the permeability with subsequently lyses of cell to inhibit cell growth. Gunalana et al. (2012) also explained the damage of cell membrane with leakage of protein, minerals and genetic material by the interaction of ZnO NPs with microbial strains.

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**Figure 1. Flow sheet diagram representing green synthesis of ZnO NPs:** (a) dried grind leave of *C. sinensis*, (b) leave extract, (c) synthesis of ZnO NPs, (d) settled ZnO NPs having supernatant layer, (e) drying of ZnO NPs at 40°C and (f) dried and calcined at 100°C ZnO NPs.

**Figure 2. Zone of inhibition (mm) measured for antimicrobial activity of Green tea ZnO NPs evaluated on (a) Staphylococcus aureus (b) Escherichia. coli (c) Aspergillus niger.**
Senthilkumar and Sivakumar (2014) reported no zone of inhibition for *E. coli*, 5.300 ± 0.570 for *S. aureus* and 3 ± 1.00 for *A. niger* against *C. sinensis* ZnO NPs. Antimicrobial activity of black tea (*C. sinensis*) extract was performed by Vasudeo and Sonika (2009) for different pathogen bacterial strains and found zone of inhibition 14 ± 2 for both *E. coli* and *S. auerus*. The MIC calculated for chloroform tea extract was 25 µg/mL. Boran et al. (2015) studied the antibacterial activity of Tea seeds against some famous pathogenic strains and reported the significant biocidal property of seeds. During this study *C. sinensis* NPs depicted better antimicrobial activity than other researchers’ findings and with low MIC. The high zone of inhibition with MIC was in agreement with documented literatures that ZnO NPs rupture lipid bilayer of bacterial and fungal cell wall with the ultimate death of microbes (Saravanakkumar et al., 2016).

### 3.2. Characterization

Some qualitative tests were performed to determine the presence or absence of important bioactive compounds in the crude leaves extract and in supernatant layer after ZnO NPs settlement; like alkaloids, flavonoids, carbohydrates, proteins, terpenes, phenols and saponins. There was strong presence of all above bioactive phytochemicals in leave extract whereas weak presence of most phytochemicals was noticed in supernatant layer. During screening proteins and phenols were absent in supernatant layer, although saponins were strongly present in both extracts. The weak presence or absence of these natural products was in agreement with reported literature (Uzunalic et al., 2006) that bioactive constituents are involved in reduction and capping of metal oxides during NPs synthesis (Malapermal et al., 2015). Absence of protein might be related with its association with ZnO NPs synthesis and stabilization (Moghaddam et al., 2017).

### 3.3. UV–visible and FTIR analysis

The spectrum obtained by UV–visible spectroscopy as shown in Figure 3 represented characteristic peak of pure ZnO NPs with absorption maxima of 350 nm. The peak broadening was between 320 and 380 nm which was in good agreement with the reported literature (Awad et al., 2014; Datta et al., 2017; Saravanakkumar et al., 2016). No other peak observed in spectrum indicating high purity and crystallinity of ZnO NPs (Santhoshkumar, Kumar, & Rajeshkumar, 2017).

Further FTIR of crude leaves extract and ZnO NPs also depicted the compatible results with other researchers’ findings and different pattern of peaks were observed in both dried moieties as shown in Figure 4.

There is a broad stretch between 3000 and 3600 cm⁻¹ with absorption maxima at 3320 cm⁻¹ that ascribed the stretching frequencies of amino and hydroxyl of alcohols and phenols. An absorption peak at 2910 cm⁻¹ represented the symmetric and asymmetric stretching of aliphatic functional group (CH₃ and CH₂). When these two peaks are compared with the IR- spectrum of ZnO NPs, these stretching became narrow with decrease of peak broadening in NPs spectrum might be associated that these functional groups are used to reduce Zn²⁺ into its oxide. Further when both spectrums compared it was revealed to have visible difference between absorption maxima and

| Microbial strain         | Aq. extract of *Camellia sinensis* leaves (100 mg/mL) | ZnO NPs of *Camellia sinensis* leaf (100 mg/mL ± SD) | Gentamycin (std. antibiotic) (100 mg/mL) | MIC ± SD |
|--------------------------|------------------------------------------------------|---------------------------------------------------|----------------------------------------|----------|
| *Staphylococcus aureus*  | No zone                                              | 40.05 mm ± 0.137                                   | 25 mm                                  | 9.765 µg ± 0.00 |
| *Escherichia coli*       | No zone                                              | 36.15 mm ± 0.304                                   | 26 mm                                  | 19.531 µg ± 0.00 |
| *Aspergillus niger*      | No zone                                              | 40.10 mm ± 0.05                                    | –                                      | 5000 µg ± 0.00 |

Where no. of treatments (N) = 3, standard deviation = SD.
stretched frequencies. As ZnO NPs spectrum have two prominent sharp peaks at 610 and 520 cm$^{-1}$ of C-alkyl chloride and hexagonal ZnO (Nalvolthula, Merugu, & Rudra, 2014) that is totally absent in crude leaves extract spectrum. Moreover two weak peaks at 1650 cm$^{-1}$ (carbonyl functional group in amide I and II) and 1430 cm$^{-1}$ (C–N stretching frequencies of amide I and –CH$_2$– scissoring vibrations of proteins) appeared prominent and sharp in NPs spectrum. These results are in agreement with reported findings that proteins stabilize the NPs and also justified the absence of protein in supernatant layer of ZnO NPs during phytochemical studies. Two prominent peaks at 1017 and 990 cm$^{-1}$ corresponded to C–O vibrational stretching frequencies of alcohol and amino acids (Salem et al., 2016) and C–N stretch of amine respectively found in crude leaves spectrum whereas two weak peaks appeared at 1050 and 960 cm$^{-1}$ in ZnO NPs.

Figure 3. UV-visible spectrum of green tea ZnO NPs showing characteristic absorption between 320 and 380 nm and $\lambda_{\text{max}}$ at 350 nm.

Figure 4. FTIR of crude dried extract of Camellia sinensis and its ZnO NPs representing characteristic functional groups peaks.
spectrum. The presence of some sharp and prominent peaks in crude extract spectrum and absence or weak presence in ZnO NPs spectrum suggested that those functional groups performing the job of capping, dispersing and stabilizing agents for NPs.

3.4. XRD studies

The spectrum of green synthesized ZnO NPs is given in Figure 5(b) and observed prominent peaks that were in fair agreement with reported literature of international Centre of Diffraction Data card (JCPDS-36–1451). Whereas diffractogram of green tea leaves extract (Figure 5a) depicted no such peak pattern was found in ZnO NPs, moreover no characteristic peaks of ZnO was noticed. Both spectrums were quite different and revealed presence of ZnO NPs formation in Figure 5(b) after reacting extract with Zinc acetate. The peaks in ZnO NPs spectrum were appeared with 2θ ranging from 20 to 70 with noticeable diffraction angles of 23.2°, 24.1°, 26.2°, 28.1°, 32.2°, 34.45°, 36.29°, 46.22°, 55.19° and 67.15° that indexed size of crystals with prominent indices plane of ZnO (hkl) as (100), (002), (101), (102), (110) and (112). Thus average size of NPs was ranged between 30 and 40 nm, calculated by Debye–Scherer equation (Saravanakkumar et al., 2016). These results of XRD were compatible with reported researchers’ work that confirmed the prepared ZnO sample is highly crystalline, having hexagonal wurtzite crystalline structure calculated by Bragg equation, \( \lambda = 2d \sin \theta \) (Agarwal et al., 2017). The typical XRD pattern revealed that the sample contains zinc oxide nanoparticles. XRD pattern of synthesized metal oxide nanoparticles showed a high crystallinity of sample level with diffraction angles which correspond to the characteristic face centred cubic. XRD patterns were analysed to determine peak intensity, position and width (Moghaddam et al., 2017). Vennila, Jesurani, Priyadharshini, and Ranjani (2016) also reported similar results of XRD Diffraction with diffractions angles at 23.4°, 27.9°, 35.3° and 44.3°; indexed (111), (220), (101) and (311) plane that corresponded to face centred cubic ZnO plane. Moreover many researchers found XRD diffractogram in good agreement with data cards JCPDS-36–1451 (Awwad et al., 2014; Saravanakkumar et al., 2016), JCPDS-5–0566 (Vidya et al., 2013), JCPDS-89–1397 (Joel & Badhusha, 2016) and JCPDS-01–079- 0207 ((Nalvolthula et al., 2014).

3.5. SEM analysis

The images gained by SEM analysis represented the morphologies of green tea mediated resultant ZnO nanosheets; as Figure 6(a) showed the surface image of primary particles merged together to yield bigger sized secondary particles. Although larger quantities of phytochemicals in leaves extract synthesized nanosheets with quite surface; Figure 6(b–d) demonstrated pretty dense morphology of nanoflowers randomly oriented overlapping. Several thinner sheets aggregated to form nanosheet networks, where individual sheets seem to have lateral dimension of less than 1 μm. The present results of SEM are in good agreement with Awwad et al. (2014) documented green synthesized ZnO nanosheets and nanoflowers with average size 500 nm and average thickness of 8 nm. Whereas Saravanakkumar et al. (2016) found the images of nanostructures that are highly aggregated, irregular as well as uniform hexagonal plates. Datta et al. (2017) revealed biosynthesized NPs’ SEM results as irregular structure of radical, cylindrical and spherical particles aggregated in small cluster.
4. Conclusion
Thus ZnO nano particles were synthesized by using green tea leaves extract that effectively inhibit microbial growth. Moreover during characterization UV–visible spectral peak at 350 nm confirmed the purity of ZnO NPs and FTIR results documented clearly the capping, reducing and stabilizing phytochemicals found in green tea. XRD diffractogram revealed characteristic peak of ZnO NPs with size range 30–40nm those coalesced to organize in nanosheets as depicted in SEM images.

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References
Agarwal, H., Kumar, S. V., & RajeshKumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles– An eco-friendly approach. Resource-Efficient Technologies, 3, 406–413. doi:10.1016/j. refit.2017.03.002
Awad, A. M., Albiss, B., & Ahmad, A. L. (2016). Green synthesis, characterization and optical properties of zinc oxide nanosheets using Olea europea leaf extract. Advanced Materials Letters, 5, 520–524. doi:10.5185/amlett.2014.5575
Borah, H., Ciftci, C., Er, A., Kose, O., Kurtoglu, I. Z., & Kays, S. (2015). Evaluation of antibacterial activity of green tea (Camellia sinensis L.) seeds against some fish pathogens in rainbow trout (Oncorhynchus mykiss, Walbaum). Turkish Journal of Fisheries and Aquatic Sciences, 15, 49–57. doi:10.4194/1303-2712-v15_1_06
Datta, A., Putra, C., Bharadwaj, H., Kaur, S., Dimri, N., & Kharuria, R. (2017). Green synthesis of zinc oxide nanoparticles using parthenium hysterophorus leaf extract and evaluation of their antibacterial properties. Journal of Biotechnology and Biomaterials, 7, 271–275. doi:10.4172/2155-952X.1000271
Dhanemazhi, A. C., Rajeswari, V., & Sathiyajothi, S. (2017). Green synthesis of zinc oxide nanoparticle using green tea leaf extract for supercapacitor application. Materials Today: Proceedings, 4, 660–667. doi:10.1016/j.matpr.2017.01.070
Femi, V., Prabha, P. H., Sudha, P., Devibala, B., & Jerald, A. L. (2011). Anti bacterial effect of ZnO-Au nanocomposites. International Journal of Biotechnology Engineering, 1, 1–8.
Geraldes, A. N., Da Silva, A. A., Leal, J., Estrado-Villegas, G. M., Linconap, N., Katti, K. V., & Lug & Atidleo, A. B. (2016). Green nanotechnology from plant extracts: synthesis and characterization of gold nanoparticles. Advances in Nanoparticles, 5, 176–185. doi:10.4236/anp
Gunalana, S., Sivaraja, R., & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International, 22, 693–700. doi:10.1016/j.pnsc.2012.11.015
Hasan, M. F., Das, R., Khan, A., Hassan, M. S., & Rahman, M. (2009). The determination of antibacterial and anti-fungal activities of polygonum hydropiper (L) Root extract. Advances in Biological Research, 3, 53–56.
Joel, C., & Badhusha, M. S. M. (2016). Green synthesis of ZnO nanoparticles using phyllanthus emblica stem extract and their antibacterial activity. Der Pharma Chemica Lettre, 8, 218–223.
Malapermal, V., Mbathe, J. N., Gengan, R. M., & Anand, K. (2015). Biosynthesis of biometallic Ag- Au nanoparticles Ocimum basilicum (L) with antibiotic and antimicrobial properties. Advanced Materials Letters, 6, 1050–1057. doi:10.5185/aml.2015.5997
Mishra, C. K., Sasmal, D., & Shrivastava, B. (2012). An in vitro evaluation of the. Anthelmintic activity of unripe fruits extract of Carissa carandas Linn. International Journal of Drug Development and Research, 4, 393–397.
Mishra, V., & Sharma, R. (2015). Green synthesis of zinc oxide nanoparticles using fresh peels extract of punica granatum and its antimicrobial activities. International Journal of Pharma Research and Health Sciences, 3, 694–699.
Mohgaddam, A. B., Moniri, M., Azizi, S., Rahim, R. A., Arif, A. B., Saad, W. Z., ... Mohamad, R. (2017). Biosynthesis of ZnO nanoparticles by a new pichia kudriavzevi yeast strain and evaluation of their antimicrobial and antioxidant activities. Molecules, 22, 872–890. doi:10.3390/molecules22060872
Nalvolthula, R., Merugu, R., & Rudra, M. P. (2014). Phytochemical analysis, synthesis, antitumor and antimicrobial activity of Silver Nanoparticles using flower extracts of Ixora coccinea. International Journal of ChemTech Research, 7, 2337–2340.
Pattanaoyak, M., & Nayok, P. L. (2013). Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract. International Journal of Plant, Animal and Environmental Sciences, 3, 68–78.
Rani, R., Nagpal, D., Gullaiya, S. Modan, S., & Agrawal, S. S. (2016). Review article phytochemical, pharmacological and beneficial effects of green tea. International Journal of Pharmacognosy and Phytochemical Research, 6, 420–426.
Saleem, N. M., Alibanna, L. S., & Awad, A. M. (2016). Green synthesis of sulfur nanoparticles using Punica granatum peels and the effects on the growth of tomato by foliar spray applications. Environmental Nanotechnology, Monitoring & Management, 6, 83–87. doi:10.1016/j.enmm.2016.06.006
Santhoshkumar, J., Kumar, S. V., & Rajeshkumar, S. (2017). Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. Resource Efficient Technologies, 3, 1–7. doi:10.1016/j.reffit.2017.05.001
Saravankumar, D., Sivarajani, S., Umanaheswari, M., Pandiarajan, S., & Ravikumar, B. (2016). Green synthesis of ZnO nanoparticles using trachyspermum ammi seed extract for antibacterial investigation. Der Pharma Chemica, 8, 173–180.
Senthilkumar, S. R., & Sivakumar, T. (2014). Green tea (Camellia sinensis) mediated synthesis of Zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. International Journal of Pharmacy and Pharmaceutical Sciences, 6, 461–465.
Sundararajon, A., Ambika, S., & Bharathri, K. (2015). Plant extract mediated synthesis of ZnO nanoparticles using Pongamia pinnata and their activity against pathogenic bacteria. Advanced Powder Technology, 26, 1294–1299. doi:10.1016/j.apt.2015.07.001
Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. Internationale Pharmaceutica Scienica, 1, 98–106.
Uzunsic, P., Skerget, A., Knez, M., Weinreich, Z., Otto, B., & Gruner, S. (2006). Extraction of active ingredients from green tea. (Camellia sinensis): Extraction efficiency of major catechins and caffeine. Food Chemistry, 96, 597–605. doi:10.1016/j.foodchem.2005.03.015
Vasudeo, Z., & Sonika, B. (2009). Antimicrobial activity of tea (Camellia sinensis). Biomedical & Pharmacology Journal, 2, 173–175.
Vennila, S., Jesurani, S. S., Priyadarshini, M., & Ronjani, M. (2016). Eco-friendly synthesis of metal oxide nanoparticles using Carissa carandas fruit extract. World Journal Pharmaceutical Research, 5, 806–812.
Vidy, C., Hirematha, S., Chandraprabha, M. N., Antonyaj, M. A. L., Gopal, I. V., Jain, A., & Bansal, K. (2013). Green synthesis of ZnO nanoparticles by Calotropis Gigantea. Proceedings of National Conference on “Women in Science & Engineering” (NCWSE 2013), SDMCET Dharwad. International Journal of Current Engineering and Technology, 118–120.

Zhang, L., Ding, Y., Povey, M., & York, D. (2008). ZnO nanofluids – A potential antibacterial agent. Progress in Natural Science, 18, 939–944. doi: 10.1016/j.pnsc.2008.01.026