

ANTI-INFLAMMATORY ACTIVITY OF BOERHAVIA DIFFUSA ZINC OXIDE NANOPARTICLE

RUNNING TITLE: Anti Inflammatory activity of boerhavia diffusa zinc oxide - a nanoparticle study

ABSTRACT:

INTRODUCTION:

Boerhaavia diffusa is a species of flowering plant in the four o'clock family which is commonly known as punarnava, red spiderling, spreading hogweed, or tarvine. It is taken in herbal medicine for pain relief and other uses. Zinc oxide is used to treat or prevent minor skin irritations such as burns, cuts, and diaper rash. Some products may be used as sunscreen.

MATERIALS AND METHODS:

Bovine serum albumin was used for the assay. 2ml of bovine albumin was mixed with 400micro microliter of hyaluronic acid mediated zinc nanoparticles in different concentrations. Diclofenac sodium in different concentrations was used as standard and then incubated for 55 c for 20 min and then the result was analysed spectrometrically.

RESULTS:

According to the results, at 10 μ l, 20 μ l ,30 μ l , 40 μ l and 50 μ l concentration the percentage of inhibition is 25%, 50%, 75%, 85%, 90%, when compared to standard concentration and its percentage of inhibition is 95% . As the concentration increases, the percentage of inhibition also increases.

CONCLUSION:

From the above study, it is evident that zinc sulphate nanoparticles synthesised by Boerhevia diffusa based showed a potent anti-inflammatory effect.

INTRODUCTION:

Boerhavia diffusa, commonly called hogweed, is known as erimiri by the Ibos of southeastern Nigeria. The leaves are cooked and eaten as vegetables, The plant is used as folk medicine to treat convulsions and as a mild laxative .(1),(2) The roots and leaves are considered to have an expectorant action, to be emetic and diuretic in large doses and are used in the treatment of asthma(3),(4). The roots of Boerhavia diffusa possess diuretic action , anti-inflammatory, antifibrinolytic , anti- convulsant and hepatoprotective activities(5),(6).Its leaf extract has hypoglycemic effects .The effects of B. diffusa leaf extract on antioxidant status in liver and kidney of alloxan diabetic rats are reported. Boerhavia diffusa comes under the family Nyctaginaceae and is considered as a tropical plant commonly seen in swampy areas in Nigeria, India and other parts of the world in both dry and rainy seasons(7),(8). The local population of Nigeria uses extracts of the crushed soaked leaves of boerhavia diffusa in the management of diabetes. B. diffusa aqueous root extract has been reported to show marked protection against thioacetamide- induced hepatic injury maintaining the various liver enzymes and serum bilirubin(9) (10) . The liquid extract of thinner roots of Boerhavia diffusa is used in traditional medicine for inflammatory disorders, bacterial infections and heart diseases.It is also used in the treatment of elephantiasis, night blindness , corneal ulcers , various hepatic disorders and as an antiviral agent(11). About forty species are distributed in tropical, subtropical and temperate regions. Among these, six species are reported in India and Boerhavia diffusa is indigenous B. diffusa is described as Punarnava by an Indian system of medicine, Ayurveda (12). Roots and whole plants of Boerhavia diffusa are used in the Ayurvedic system of medicine in Arabian countries and even today many tribal communities in India still use it for the treatment of jaundice and various other liver disorders(13). It has anti-inflammatory, diuretic, fibrinolytic, anticonvulsant properties and is also used as carminatives . Boerhavia diffusa , a well- known indigenous medicinal plant, also known as pun- arnava, has pleiotropic medicinal properties and is also a main ingredient of many ayurvedic formula- tions for the treatment of jaundice, inflammation, oedema, hypertension etc(14),(15). This plant is also being used as a green leafy vegetable in different parts of Asia and Africa due to its nutraceutical properties. It is rich in sources of minerals, vitamins and carbohydrates. It contains a large amount of compounds such

as alkaloids, rotenoids, flavonoids, amino acids, lignans, saponins, β -sitosterols and tetra-cosanoic, eicosanoic, stearic and ursolic acids(16).

Nanostructured Zinc oxide materials have received broad attention due to their distinguished performance in electronics, optics and photonics(17),(18). From the past years, synthesis of Zinc oxide thin films has been an active field because of their applications as sensors, transducers and catalysts. In the last few decades, especially since the nanotechnology initiative led by the US, study of one-dimensional materials has become a leading edge in nanoscience and nanotechnology(19),(20). With reduction in size, novel electrical, mechanical, chemical and optical properties are introduced, which are largely believed to be the result of surface and quantum confinement effects(21),(22). Nanowire-like structures are the ideal system for studying the transport process in one-dimensionally confined objects, which are of benefit not only for understanding the fundamental phenomena in low dimensional systems, but also for developing new generation nanodevices with high performance(23)(24). Zinc oxide is a key technological material. The lack of a centre of symmetry in wurtzite, combined with large electromechanical coupling, results in strong piezoelectric and pyroelectric properties and the consequent use of Zinc oxide in mechanical actuators and piezoelectric sensors(25)(26). In addition, Zinc oxide is a wide band-gap compound semiconductor that is suitable for short wavelength optoelectronic applications. The high exciton binding energy of Zinc oxide crystal can ensure efficient excitonic emission at room temperature and room temperature ultraviolet luminescence has been reported in disordered nanoparticles and thin films(27)(28). Zinc oxide is transparent to visible light and can be made highly conductive by doping.(29)

MATERIALS AND METHODS:

Plant extract preparation;

The fresh leaves of *Boerhavia diffusa* were collected in an unbiased manner and sampling was done by Randomised sampling method and washed thoroughly with distilled water. From clean and dried plant leaves fine powder was made with a homogenizer, only dried leaves of *Boerhavia diffusa* were included and other parts of the plant such as stem, root, flower were excluded in this study. About 1gm of clean dried leaves of *Boerhavia diffusa* was added to 50 ml of distilled water it was allowed to dissolve the weighted extract in conical flasks and mixed well. This mixture is boiled at 60 degrees Celsius for 7 minutes with the help of a heating mantle. Then the boiled extract is filtered with the help of filter paper.

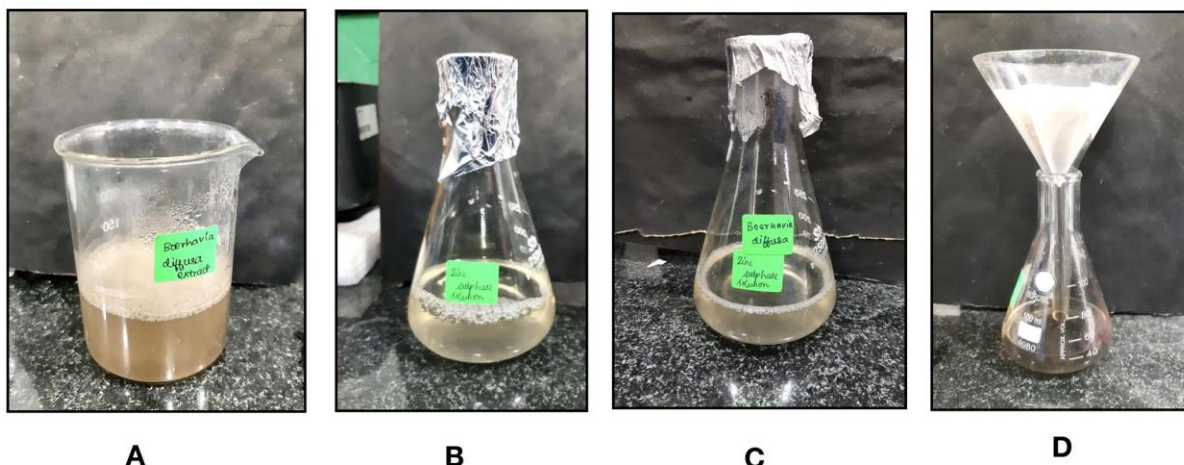


Fig 1: A denotes *Boerhavia diffusa* extract, B denotes the zinc sulphate solution, C denotes *boerhavia diffusa* and zinc sulphate solution and D denotes the the boiled extract getting filtered in filter paper

Synthesis of copper Nanoparticles:

This *Boerhavia diffusa* is treated with 0.507g of zinc sulphate and 90 ml of distilled water and it is placed in a semi-automatic shaker at 900 rpm. With the help of a double beam U-V spectrophotometer, the synthesis of nanoparticles for every one hour is noted. Then this formulation is placed in a centrifuge for 10 minutes. Now the synthesized nanoparticles which are settled at the bottom are collected. The randomized sampling method was done in an unbiased manner.

Anti Inflammatory activity:

BSA assay:

2ml of bovine albumin was mixed with 400 micro microliter of zinc nanoparticles in different concentrations. Diclofenac sodium in different concentrations was used as standard and then incubated for 55 degree celcius for 20 min and then the result was analysed spectrometrically.

RESULTS:

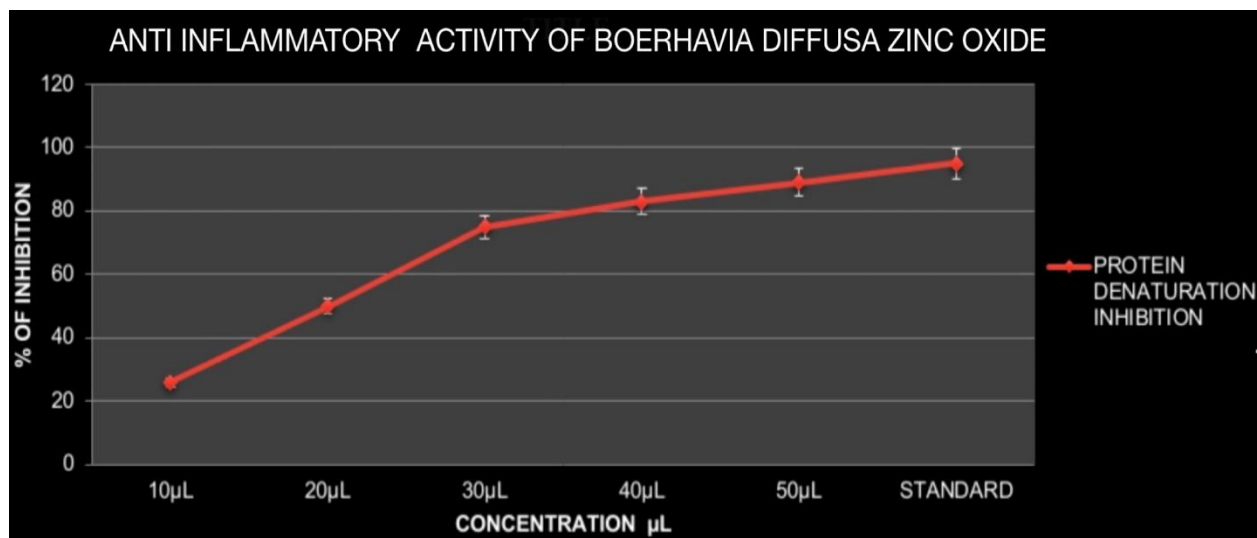


Figure 2: The graph represents the percentage of inhibition on y axis and concentration on x axis, The red line denotes the protein denaturation inhibition, data implies as mean \pm SEM

According to the results, at 10 μL the percentage of inhibition is 25% at 20 μL the percentage of inhibition is 50% at 30 μL the percentage of inhibition is 75% and at 40 μL the percentage of inhibition is 85% and at 50 μL concentration the percentage of inhibition is 90%, when compared to standard concentration its percentage of inhibition is 95%. As the concentration increases, the percentage of inhibition also increases.

DISCUSSION:

The results obtained in this study indicate that the Punarnavasava, a formulation mainly containing *B. diffusa* shows anti-inflammatory. The plant has gained a lot of importance in the field of phytochemistry because of its various pharmacological and biological activities such as immunomodulatory effects, immunosuppressive activity, anti-metastatic activity, antioxidant activity, antidiabetic activity, antiproliferative and antiestrogenic activity, analgesic and anti-inflammatory activity, antibacterial activity, anti-stress and adaptogenic activity, anti-lymphoproliferative activity, nitric oxide scavenging activity, hepatoprotective activity, antiviral activity, bronchial asthma, anti-fibrinolytic activity, chemopreventive action, genetic diversity analysis, anticonvulsant activity (30)(31). They can also improve the condition of diabetes as indicated by parameters like body weight along with serum cholesterol and triglyceride levels (32)(33). The number of functionally intact β -cells in the islet organ is of decisive importance for the development course and outcome of diabetes (34) (35). Potent antibacterial activity against gram positive and gram negative bacteria shown by the leaves of *B. diffusa* might be due to the phytochemicals present in the leaves (36)(37). *Boerhaavia diffusa* extracts exhibited

a strong inhibitory effect on the proliferation of human breast cancer cells in vitro and the antiestrogenic effects are mediated by ER (38).

Zinc oxide is a very promising material for semiconductor device applications . It has a direct and wide band gap in the near-UV spectral region , and a large free-exciton binding energy so that excitonic emission processes can persist at or even above room temperature(39)(40). Its properties have been studied since the early days of semiconductor electronics ,but the use of Zinc oxide as a semiconductor in electronic devices has been hindered by the lack of control over its electrical conductivity(41)(42). Zinc oxide crystals are almost always n-type, the cause of which has been a matter of extensive debate and research .Over the past decade we have witnessed a significant improvement in the quality of Zinc oxide single-crystal substrates and epitaxial films(43)(44).This, in turn, has led to a revival of the idea of using Zinc oxide as an optoelectronic or electronic material in its own right(45)(46). Zinc oxide, with its unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability, is a multifunctional material (47). In materials science, zinc oxide is classified as a semiconductor whose covalence is on the boundary between ionic and covalent semiconductors(48)(49). A broad energy band ,high bond energy and high thermal and mechanical stability at room temperature make it attractive for potential use in electronics, optoelectronics and laser technology (50)(51).The piezo- and pyroelectric properties of Zinc oxide mean that it can be used as a sensor, converter, energy generator and photocatalyst in hydrogen production .Because of its hardness, rigidity and piezoelectric constant it is an important material in the ceramics industry, while its low toxicity, biocompatibility and biodegradability make it a material of interest for biomedicine and in pro-ecological systems (52)(53).Our team has extensive knowledge and research experience that has translate into high quality publications(54–73) (74) (75) (76) (77) (78) (79) (80) (81) (82) (83) (84) (85) (86) (87)

CONCLUSION:

From the above study, it is evident that zinc sulphate nanoparticles synthesised by Boerhevia diffusa based showed a potent anti-inflammatory effect(88-97). It can be used as a potent antifungal agent against fungal infections in the form of gels for external use and in the form of mouthwashes to control oral thrush .Also further studies can be done to assess it's antioxidant , antibacterial bacterial and cytotoxic activity which could be used in the treatment of numerous diseases.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES:

1. Gharate M, Kasture V. Evaluation of anti-inflammatory, analgesic, antipyretic and antiulcer activity of Punarnavasava: an Ayurvedic formulation of Boerhavia diffusa [Internet]. Oriental Pharmacy and Experimental Medicine. 2012. Available from: <http://dx.doi.org/10.1007/s13596-012-0081-3>
2. Shankar SB, Barani Shankar S, Arivarasu L, Rajeshkumar S. Biosynthesis of Hydroxy Citric Acid Mediated Zinc Nanoparticles and Its Antioxidant and Cytotoxic Activity [Internet]. Journal of Pharmaceutical Research International. 2020. p. 108–12. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2630845>
3. Rajagopal RR. Investigation of in-vitro anthelmintic activity of ethanolic leaf extract of Boerhavia diffusa (Nyctaginaceae) including pharmacognostical and phytochemical screening [Internet]. Vol. 7, Journal of Pharmacy Research. 2013. p. 774–80. Available from: <http://dx.doi.org/10.1016/j.jopr.2013.08.009>
4. Karthik V, Arivarasu L, Rajeshkumar S. Hyaluronic Acid Mediated Zinc Nanoparticles against Oral Pathogens and Its Cytotoxic Potential [Internet]. Journal of Pharmaceutical Research International. 2020. p. 113–7. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930716>
5. Nimisha P, Assistant professor of Zoology, PSMO college, Tirurangadi, 676306. EVALUATION OF INSECTICIDAL ACTIVITY OF BOERHAVIA DIFFUSA AGAINST CERTAIN STORED PRODUCT INSECTS [Internet]. Vol. 7, International Journal of Advanced Research. 2019. p. 1252–5. Available from: <http://dx.doi.org/10.21474/ijar01/9618>

6. Shree MK, Kavya Shree M, Arivarasu L, Rajeshkumar S. Cytotoxicity and Antimicrobial Activity of Chromium Picolinate Mediated Zinc Oxide Nanoparticle [Internet]. *Journal of Pharmaceutical Research International*. 2020. p. 28–32. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2030726>
7. Švecová E, Colla G, Crinò P. Antifungal activity of *Boerhavia diffusa* L. extract against *Phytophthora* spp. in tomato and pepper [Internet]. Vol. 148, *European Journal of Plant Pathology*. 2017. p. 27–34. Available from: <http://dx.doi.org/10.1007/s10658-016-1065-9>
8. Jaisankar AI, Arivarasu L. Free Radical Scavenging and Anti-Inflammatory Activity of Chlorogenic Acid Mediated Silver Nanoparticle [Internet]. *Journal of Pharmaceutical Research International*. 2020. p. 106–12. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930715>
9. Bairwa K, Jachak SM. Anti-inflammatory potential of a lipid-based formulation of a rotenoid-rich fraction prepared from *Boerhavia diffusa*. *Pharm Biol*. 2015 Aug;53(8):1231–8.
10. Karthik V, Arivarasu L, Leslie S. Viruses and Their Treatment - A Review [Internet]. Vol. 11, *Journal of Complementary Medicine Research*. 2020. p. 121. Available from: <http://dx.doi.org/10.5455/jcmr.2020.11.02.18>
11. Saraswati S, Alhaider AA, Agrawal SS. Punarnavine, an alkaloid from *Boerhavia diffusa* exhibits anti-angiogenic activity via downregulation of VEGF in vitro and in vivo. *Chem Biol Interact*. 2013 Nov 25;206(2):204–13.
12. Ujowundu CO, Igwe CU, Enemor VHA, Nwaogu LA, Okafor OE. Nutritive and Anti-Nutritive Properties of *Boerhavia diffusa* and *Commelina nudiflora* Leaves [Internet]. Vol. 7, *Pakistan Journal of Nutrition*. 2007. p. 90–2. Available from: <http://dx.doi.org/10.3923/pjn.2008.90.92>
13. Gujar K. IN VITRO ANTIMICROBIAL POTENTIAL OF BOERHAVIA DIFFUSA LEAF EXTRACT ON PATHOGENIC ORGANISM [Internet]. *World Journal of Pharmaceutical Research*. 2017. p. 1248–56. Available from: <http://dx.doi.org/10.20959/wjpr20175-8447>
14. Gupta J, Ali M. ChemInform Abstract: Chemical Constituents of *Boerhavia diffusa* Linn. roots [Internet]. Vol. 30, *ChemInform*. 2010. p. no – no. Available from: <http://dx.doi.org/10.1002/chin.199909221>
15. Herbal Sources Used by The Public Against Infections [Internet]. Vol. 12, *International Journal of Pharmaceutical Research*. 2020. Available from: <http://dx.doi.org/10.31838/ijpr/2020.sp1.015>
16. Shivanand G, Dole RG. AN ANALYTICAL REVIEW OF PUNARNAVA (BOERHAVIA DIFFUSA) WITH RESPECT TO CHAKSHUSHYA PROPERTY [Internet]. *INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH*. 2020. p. 1–3. Available from: <http://dx.doi.org/10.36106/ijsr/0634037>
17. Feng ZC. *Handbook of Zinc Oxide and Related Materials: Volume One, Materials*. CRC Press; 2012. 446 p.
18. Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using *Cissus vitiginea* and its antioxidant and antibacterial activity against urinary tract infection pathogens [Internet]. Vol. 48, *Artificial Cells, Nanomedicine, and Biotechnology*. 2020. p. 1153–8. Available from: <http://dx.doi.org/10.1080/21691401.2020.1817053>

19. Awasthi K. Nanostructured Zinc Oxide: Synthesis, Properties and Applications. Elsevier; 2020. 525 p.
20. M G, Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, et al. Phyllanthus reticulatus mediated synthesis and characterization of silver nanoparticles and its antibacterial activity against gram positive and gram negative pathogens [Internet]. Vol. 10, International Journal of Research in Pharmaceutical Sciences. 2019. p. 3099–106. Available from: <http://dx.doi.org/10.26452/ijrps.v10i4.1603>
21. Morkoç H, Özgür Ü. Zinc Oxide: Fundamentals, Materials and Device Technology. John Wiley & Sons; 2008. 488 p.
22. Sivaraj R, Pattanathu K S, Rajiv P, Narendhran S, Venckatesh R. Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity [Internet]. Vol. 129, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014. p. 255–8. Available from: <http://dx.doi.org/10.1016/j.saa.2014.03.027>
23. Ellmer K, Klein A, Rech B. Transparent Conductive Zinc Oxide: Basics and Applications in Thin Film Solar Cells. Springer Science & Business Media; 2007. 446 p.
24. Niveditha AS, Sankari Niveditha A, Geetha RV, Arivarasu L. Will Alternative Medicine Help Us to Fight Against COVID-19 [Internet]. International Journal of Current Research and Review. 2020. p. 112–6. Available from: <http://dx.doi.org/10.31782/ijcrr.2020.sp47>
25. Willander M. Zinc Oxide Nanostructures: Advances and Applications. CRC Press; 2014. 232 p.
26. Akash N, Arivarasu L, Rajeshkumar S. Anti-inflammatory and Antioxidant Potential of Hyaluronic Acid Mediated Zinc Nanoparticles [Internet]. Journal of Pharmaceutical Research International. 2020. p. 33–7. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2030727>
27. Litton CW, Collins TC, Reynolds DC. Zinc Oxide Materials for Electronic and Optoelectronic Device Applications. John Wiley & Sons; 2011. 386 p.
28. Aathira CM, Arivarasu L, Rajeshkumar S. Antioxidant and Anti-Inflammatory Potential of Chromium Picolinate Mediated Zinc Oxide Nanoparticle [Internet]. Journal of Pharmaceutical Research International. 2020. p. 118–21. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930717>
29. Jagadish C, Pearton SJ. Zinc Oxide Bulk, Thin Films and Nanostructures: Processing, Properties, and Applications. Elsevier Science; 2006. 600 p.
30. Boerhavia diffusa Linn [Internet]. SpringerReference. Available from: http://dx.doi.org/10.1007/springerreference_68165
31. Devaraj E, Roy A, Veeraragavan GR, Magesh A, Sreeba AV, Arivarasu L, et al. β -Sitosterol attenuates carbon tetrachloride-induced oxidative stress and chronic liver injury in rats [Internet]. Vol. 393, Naunyn-Schmiedeberg's Archives of Pharmacology. 2020. p. 1067–75. Available from: <http://dx.doi.org/10.1007/s00210-020-01810-8>
32. Khare CP. Boerhavia diffusa Linn [Internet]. Indian Medicinal Plants. 2007. p. 1–1. Available from: http://dx.doi.org/10.1007/978-0-387-70638-2_228
33. Mohammadi Z, Abbott PV. Antimicrobial substantivity of root canal irrigants and medicaments: A

review [Internet]. Vol. 35, Australian Endodontic Journal. 2009. p. 131–9. Available from: <http://dx.doi.org/10.1111/j.1747-4477.2009.00164.x>

34. Akinnibosun FI, Akinnibosun HA, Ogedegbe D. Investigation on the antibacterial activity of the aqueous and ethanolic extracts of the leaves of *Boerhavia diffusa* L [Internet]. Vol. 4, Science World Journal. 2010. Available from: <http://dx.doi.org/10.4314/swj.v4i2.51839>
35. Website [Internet]. [cited 2021 Mar 13]. Available from: 15. Balraj S, Aiyavu C, Manivasagam T, Kalaimathi J, Rajeshkumar S. Neuroprotective Role of Diosmin on Rotenone Induced Neurotoxicity in SH-SY5Y Neuroblastoma Cells. *Biosc.Biotech.Res.Comm.* 2020;13(4). 1782-1787. DOI: <http://dx.doi.org/10.21786/bbrc/13.4/20>
36. Wang Y. Anti-tumor activity of *Hedyotis diffusa* Willd. in mice [Internet]. Vol. 22, Journal of Chinese Pharmaceutical Sciences. 2013. Available from: <http://dx.doi.org/10.5246/jcps.2013.02.039>
37. Nasim I, Kamath K, Rajeshkumar S. Evaluation of the re-mineralization capacity of a gold nanoparticle-based dental varnish: An in vitro study [Internet]. Vol. 23, Journal of Conservative Dentistry. 2020. p. 390. Available from: http://dx.doi.org/10.4103/jcd.jcd_315_20
38. G S, Saurabh G, Komal S. Comparative Characterization for Antimicrobial Activity and Bioactive Compounds Present in Leaf Extract of *Ocimum sanctum* [Internet]. Vol. 03, Journal of Food & Industrial Microbiology. 2018. Available from: <http://dx.doi.org/10.4172/2572-4134.1000121>
39. Zhao Z, Ryu H, Lee H, Kwon SJ, Cho E-S. A Study on Formation of Aluminum-Doped Zinc Oxide Films by Pulsed-Direct Current Sputtering for CIGS Solar Cells. *J Nanosci Nanotechnol.* 2021 Sep 1;21(9):4632–7.
40. S SK, Satheesha KS. In-Vitro Antibacterial Activity of Black Tea (*Camellia sinensis*) Mediated Zinc Oxide Nanoparticles Against Oral Pathogens [Internet]. Vol. 13, Bioscience Biotechnology Research Communications. 2020. p. 2077–80. Available from: <http://dx.doi.org/10.21786/bbrc/13.4/66>
41. Wang X, Chen X, Xu G, Li J, Guo J, Wang Q. Performance of zinc oxide quantum dots coated paper and application of fluorescent anti-counterfeiting. *Appl Opt.* 2021 Mar 10;60(8):2304–13.
42. Rajeshkumar S, Malarkodi C, Al Farraj DA, Elshikh MS, Roopan SM. Employing sulphated polysaccharide (fucoidan) as medium for gold nanoparticles preparation and its anticancer study against HepG2 cell lines [Internet]. Vol. 26, Materials Today Communications. 2021. p. 101975. Available from: <http://dx.doi.org/10.1016/j.mtcomm.2020.101975>
43. Hsieh G-W, Ling S-R, Hung F-T, Kao P-H, Liu J-B. Enhanced piezocapacitive response in zinc oxide tetrapod-poly(dimethylsiloxane) composite dielectric layer for flexible and ultrasensitive pressure sensor. *Nanoscale* [Internet]. 2021 Mar 9; Available from: <http://dx.doi.org/10.1039/d0nr06743a>
44. Shunmugam R, Balusamy SR, Kumar V, Menon S, Lakshmi T, Perumalsamy H. Biosynthesis of gold nanoparticles using marine microbe (*Vibrio alginolyticus*) and its anticancer and antioxidant analysis [Internet]. Vol. 33, Journal of King Saud University - Science. 2021. p. 101260. Available from: <http://dx.doi.org/10.1016/j.jksus.2020.101260>
45. Shandiz SAS, Sharifian F, Behboodi S, Ghodrattpour F, Baghbani-Arani F. Evaluation of Metastasis Suppressor Genes Expression and Anti-Cancer Effects of Zinc Oxide Nanoparticles in Human Breast Cancer Cell Lines MCF-7 and T47D. *Avicenna J Med Biotechnol.* 2021 Jan;13(1):9–14.

46. Rajeshkumar S, Sherif MH, Malarkodi C, Ponnaniakajamideen M, Arasu MV, Al-Dhabi NA, et al. Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from *padina tetrastromatica* [Internet]. Vol. 1228, Journal of Molecular Structure. 2021. p. 129440. Available from: <http://dx.doi.org/10.1016/j.molstruc.2020.129440>
47. Li X, Zhang L, Wang Z, Wu S, Ma J. Cellulose controlled zinc oxide nanoparticles with adjustable morphology and their photocatalytic performances. *Carbohydr Polym.* 2021 May 1;259:117752.
48. Rodriguez JA, Campbell CT. A quantum chemical study of zinc oxide, copper/zinc oxide, cuprous oxide, and cupric oxide clusters and carbon monoxide chemisorption on zinc oxide(0001), copper zinc oxide(0001), and copper/zinc oxide(0001) surfaces [Internet]. Vol. 91, The Journal of Physical Chemistry. 1987. p. 6648–58. Available from: <http://dx.doi.org/10.1021/j100311a019>
49. Barma MD, Kannan SD, Indiran MA, Rajeshkumar S, Pradeep Kumar R. Antibacterial Activity of Mouthwash Incorporated with Silica Nanoparticles against *S. aureus*, *S. mutans*, *E. faecalis*: An in-vitro Study [Internet]. *Journal of Pharmaceutical Research International.* 2020. p. 25–33. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1630646>
50. Klingshirn CF, Waag A, Hoffmann A, Geurts J. Zinc Oxide: From Fundamental Properties Towards Novel Applications. Springer Science & Business Media; 2010. 300 p.
51. Barma MD. Synthesis of Triphala Incorporated Zinc Oxide Nanoparticles and Assessment of its Antimicrobial Activity Against Oral Pathogens : An In-Vitro Study [Internet]. Vol. 13, Bioscience Biotechnology Research Communications. 2020. p. 74–8. Available from: <http://dx.doi.org/10.21786/bbrc/13.7/14>
52. Baskoutas S. Zinc Oxide Nanostructures: Synthesis and Characterization. MDPI; 2018. 302 p.
53. Vikneshan M, Saravanakumar R, Mangaiyarkarasi R, Rajeshkumar S, Samuel SR, Suganya M, et al. Algal biomass as a source for novel oral nano-antimicrobial agent [Internet]. Vol. 27, Saudi Journal of Biological Sciences. 2020. p. 3753–8. Available from: <http://dx.doi.org/10.1016/j.sjbs.2020.08.022>
54. Rajeshkumar S, Venkat Kumar S, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells [Internet]. Vol. 117, Enzyme and Microbial Technology. 2018. p. 91–5. Available from: <http://dx.doi.org/10.1016/j.enzmictec.2018.06.009>
55. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation [Internet]. Vol. 19, Biocatalysis and Agricultural Biotechnology. 2019. p. 101138. Available from: <http://dx.doi.org/10.1016/j.bcab.2019.101138>
56. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of *Enterococcus* sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells [Internet]. Vol. 27, Environmental Science and Pollution Research. 2020. p. 8166–75. Available from: <http://dx.doi.org/10.1007/s11356-019-07511-x>
57. Website [Internet]. Available from: 4. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using *Gymnema sylvestri* leaf extract and evaluation of its antibacterial activity [Internet]. Vol. 32, South African Journal of Chemical Engineering. 2020. p. 1–4. Available from: <http://dx.doi.org/10.1016/j.sajce.2019.11.005>

58. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poures MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends [Internet]. Vol. 277, Fuel. 2020. p. 118166. Available from: <http://dx.doi.org/10.1016/j.fuel.2020.118166>
59. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea* [Internet]. Vol. 29, South African Journal of Chemical Engineering. 2019. p. 17–23. Available from: <http://dx.doi.org/10.1016/j.sajce.2019.04.001>
60. R KR, Kathiswar RR, Ezhilarasan D, Rajeshkumar S. β -Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line [Internet]. Vol. 108, Journal of Biomedical Materials Research Part A. 2020. p. 1899–908. Available from: <http://dx.doi.org/10.1002/jbm.a.36953>
61. Kirmusaoğlu S. Bacterial Pathogenesis and Antibacterial Control. BoD – Books on Demand; 2018. 154 p.
62. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells [Internet]. Vol. 38, Human & Experimental Toxicology. 2019. p. 694–702. Available from: <http://dx.doi.org/10.1177/0960327119839173>
63. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets [Internet]. Vol. 17, Hepatobiliary & Pancreatic Diseases International. 2018. p. 192–7. Available from: <http://dx.doi.org/10.1016/j.hbpd.2018.04.003>
64. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective [Internet]. Vol. 19, Arab Journal of Gastroenterology. 2018. p. 56–64. Available from: <http://dx.doi.org/10.1016/j.ajg.2018.03.002>
65. Patra C, Ahmad I, Ayaz M, Khalil AT, Mukherjee S, Ovais M. Biogenic Nanoparticles for Cancer Theranostics. Elsevier; 2021. 284 p.
66. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress [Internet]. Vol. 80, Drug Development Research. 2019. p. 714–30. Available from: <http://dx.doi.org/10.1002/ddr.21571>
67. Chaughule RS. Dental Applications of Nanotechnology. Springer; 2018. 277 p.
68. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol. 2021 Feb;122:105030.
69. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? [Internet]. Vol. 132, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2021. p. 118–9. Available from: <http://dx.doi.org/10.1016/j.oooo.2021.01.025>
70. Ezhilarasan D, Apoorva VS, Vardhan NA. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells [Internet]. Journal of Oral Pathology & Medicine. 2018. Available from: <http://dx.doi.org/10.1111/jop.12806>
71. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Sivaprakasam

- AN. Compatibility of Nonoriginal Abutments With Implants [Internet]. Vol. 28, Implant Dentistry. 2019. p. 289–95. Available from: <http://dx.doi.org/10.1097/id.0000000000000885>
72. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products [Internet]. Vol. 507, Aquaculture. 2019. p. 1–6. Available from: <http://dx.doi.org/10.1016/j.aquaculture.2019.04.004>
73. Markov A, Thangavelu L, Aravindhana S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders [Internet]. Vol. 12, Stem Cell Research & Therapy. 2021. Available from: <http://dx.doi.org/10.1186/s13287-021-02265-1>
74. Pushpaanjali G, Geetha RV, Lakshmi T. Knowledge and Awareness about Antibiotic Usage and Emerging Drug Resistance Bacteria among Dental Students. *Journal of Pharmaceutical Research International*. 2020 Aug 24;34–42.
75. Aathira CM, Geetha RV, Lakshmi T. Knowledge and Awareness about the Mode of Transmission of Vector Borne Diseases among General Public. *Journal of Pharmaceutical Research International*. 2020 Aug 24;87–96.
76. Baskar K, Lakshmi T. Knowledge, Attitude and Practices Regarding HPV Vaccination among Undergraduate and Postgraduate Dental Students in Chennai. *Journal of Pharmaceutical Research International*. 2020 Aug 25;95–100.
77. Manya Suresh LT. Wound Healing Properties of Aloe Barbadensis Miller-In Vitro Assay. *Journal of Complementary Medicine Research*. 2020;11(5):30–4.
78. First Report on Marine Actinobacterial Diversity around Madras Atomic Power Station (MAPS), India [Internet]. [cited 2021 Aug 31]. Available from: <http://alinteridergisi.com/article/first-report-on-marine-actinobacterial-diversity-around-madras-atomic-power-station-maps-india/>
79. Physicochemical Profile of Acacia Catechu Bark Extract – An in Vitro Stud - *International Journal of Pharmaceutical and Phytopharmacological Research* [Internet]. [cited 2021 Aug 31]. Available from: <https://ejppr.com/article/physicochemical-profile-of-acacia-catechu-bark-extract-an-in-vitro-stud>
80. Lakshmi T. Antifungal Activity of Ficus racemosa Ethanolic Extract against Dermatophytes-An in vitro Study. *Journal of Research in Medical and Dental Science*. 2021;9(2):191–3.
81. Awareness of Drug Abuse among Teenagers - *International Journal of Pharmaceutical and Phytopharmacological Research* [Internet]. [cited 2021 Aug 31]. Available from: <https://ejppr.com/article/awareness-of-drug-abuse-among-teenagers>
82. Mangal CSK, Anitha R, Lakshmi T. Inhibition of Nitric oxide Production and Nitric oxide Synthase Gene Expression in LPS Activated RAW 264 .7 Macrophages by Thyme oleoresin from *Thymus vulgaris*. *J Young Pharm*. 2018;10(4):481.
83. COX2 Inhibitory Activity of Abutilon Indicum - *Pharmaceutical Research and Allied Sciences* [Internet]. [cited 2021 Aug 31]. Available from: <https://ijpras.com/article/cox2-inhibitory-activity-of-abutilon-indicum>
84. Jibu RM, Geetha RV, Lakshmi T. Isolation, Detection and Molecular Characterization of

Staphylococcus aureus from Postoperative Infections. *Journal of Pharmaceutical Research International*. 2020 Aug 24;63–7.

85. Sindhu PK, Thangavelu L, Geetha RV, Rajeshkumar S, Raghunandhakumar S, Roy A. Anorectic drugs: an experimental and clinical perspective – A Review. *Journal of Complementary Medicine Research*. 2020;11(5):106–12.
86. Nivethitha R, Thangavelu L, Geetha RV, Anitha R, RajeshKumar S, Raghunandhakumar S. In Vitro Anticancer Effect of Sesamum Indicum Extract -. *Journal of Complementary Medicine Research*. 2020;11(5):99–105.
87. Mariona P, Roy A, Lakshmi T. Survey on lifestyle and food habits of patients with PCOS and obesity. *Journal of Complementary Medicine Research*. 2020;11(5):93–8.
88. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. *Pesqui Bras Odontopediatria Clin Integr*. 2019 Mar 12;19(0):4668.
89. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer’s disease. *Clin Exp Pharmacol Physiol* [Internet]. 2017 Mar [cited 2021 Sep 15];44(3). Available from: <https://pubmed.ncbi.nlm.nih.gov/28004401/>
90. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. *Case Rep Dent* [Internet]. 2019 Jan 22 [cited 2021 Sep 15];2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/30805222/>
91. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. *Oncol Rev* [Internet]. 2014 Jun 30 [cited 2021 Sep 15];8(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/25992232/>
92. Menon S, Ks SD, R S, S R, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. *Colloids Surf B Biointerfaces* [Internet]. 2018 Oct 1 [cited 2021 Sep 15];170. Available from: <https://pubmed.ncbi.nlm.nih.gov/29936381/>
93. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. *J Public Health Dent* [Internet]. 2020 Jan [cited 2021 Sep 15];80(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/31710096/>
94. Praveen K, Narayanan V, Muthusekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. *Br J Oral Maxillofac Surg* [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). Available from: <https://pubmed.ncbi.nlm.nih.gov/11286449/>
95. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. *Int Endod J* [Internet]. 2011 Jun [cited 2021 Sep 15];44(6). Available from: <https://pubmed.ncbi.nlm.nih.gov/21255047/>
96. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. *Chem Biol Interact*. 2019 Aug 1;308:206–15.

97. Kumar MS, Vamsi G, Sripriya R, Sehgal PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J Periodontol. 2006 Nov;77(11):1803-8.

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