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RESEARCH ARTICLE
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Antibacterial Activity of Zinc Oxide Nanoparticles Against Streptococcus Mutans Using Picrorhiza

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ABSTRACT

Introduction: Picrorhiza kurroa is a perennial herb belonging to the family plantaginaceae , acknowledged for its antibacterial, anti fungal, antioxidant activity etc . The antibacterial activity of Zinc oxide nanoparticles has acquired an enormous amount of interest recently

Aim: The aim of this study is to assess the antibacterial activity of zinc oxide nanoparticles against streptococcus mutans using Picrorhiza kurroa

Materials and methods: The antibacterial activity of zinc oxide nanoparticles against streptococcus mutans was done using minimum inhibitory concentration

(MIC) method . Various concentration of Picrorhiza - zinc oxide extract were tested to check the antibacterial activity of each concentration for excelling appraisal

Conclusion: To conclude that Picrorhiza kurroa possess significant antibacterial activity against streptococcus mutans

Keywords: *Antibacterial , streptococcus mutans, Picrorhiza kurroa , Zinc oxide nanoparticles , ZnO nanoparticles* .

INTRODUCTION

Popularly known as "Kutki" or "Kurro" and "Indian gentian," Picrorhiza kurroa Royle ex Benth (Family: Plantaginaceae, formerly known as Scrophulariaceae is a well-known medicinal plant. It may be found at elevations of 3000-5000 m in the north-western Himalayas.("'Picrosides' from Picrorhiza Kurroa as Potential Anti-Carcinogenic Agents" 2019) In Ayurveda, the rhizome of Picrorhiza kurroa, was used in therapeutic settings to treat hypertension and heart conditions. It is a well-known medication for treating a number of liver and spleen disorders as well as fever. The Himalayan area and China are the only places where some species are found. Despite the fact that P.kurroa is mostly found in the Western Himalaya at elevations of 3000- 4300 meters ("Phytochemical Screening and Antimicrobial Activity of Picrorrhiza Kurroa, an Indian Traditional Plant Used to Treat Chronic Diarrhea" 2016) . The Ayurvedic advantages of P. kurroa are well known and also include immune-modulating, anti-allergic, and antineoplastic activity, persistent fever, skin problems, and diabetes,("Antioxidant and Antimicrobial Activity Displayed by a Fungal Endophyte Alternaria Alternata Isolated from Picrorhiza Kurroa from Garhwal Himalayas, India" 2021). P. kurroa has a variety of medicinal potentials that might be helpful in the creation of pharmaceuticals or their precursors. Additionally, the plant enjoys a stellar reputation among local medical professionals.("Antioxidant and Anti-Neoplastic Activities of Picrorhiza Kurroa Extracts" 2011). Due to the presence of bioactive components such as picroside I and picroside II, cucurbitacins, and phenolic components, the plant possesses a variety of therapeutic characteristics.

These chemical elements are present in this herb's roots and rhizomes and are used to treat a variety of conditions, including asthma, fever, and conditions of the liver and spleen. The yearly demand for P. kurroa in the world is 500 tonnes, but the annual production is just 375 tonnes.("Picrorhiza Kurroa: A Promising Traditional Therapeutic Herb from Higher Altitude of Western Himalayas" 2020). Picrorhiza kurroa has antioxidant, anti-inflammatory, and immunomodulatory properties, but its hepatoprotective impact is what makes it so valuable. Due to faulty digestive secretions, the rhizomes have long been utilized to treat gastrointestinal issues.(Kant et al. 2013).P. kurroa also has a reputation for having a wide range of pharmacological activities, including those that are anticancer, hepatoprotective, immunomodulatory, antibacterial, antioxidant, antiallergic, and anti-asthmatic. The main groups of secondary metabolites found in P. kurroa are iridoids, terpenoids, flavonoids, phenolic acids, steroids, and their glycosides. However, due to recent overexploitation of this plant and its extensive therapeutic potential, it is now listed as one of the Himalayas' endangered medicinal plants.("A Comprehensive Phytochemical, Ethnomedicinal, Pharmacological Ecology and Conservation Status of Picrorhiza Kurroa Royle Ex Benth.: An Endangered Himalayan Medicinal Plant" 2021)

Due to their unique physical and chemical characteristics, zinc oxide nanoparticles (ZnO NPs), one of the most significant metal oxide nanoparticles, are often used in many different disciplines. Additionally, ZnO NPs have outstanding antibacterial, antimicrobial, and UV-blocking qualities.

As a result, the textile industry's final textiles with the addition of ZnO NPs displayed the desirable properties of resistance to UV and visible light, antibacterial properties, and deodorant properties (Jiang, Pi, and Cai 2018). The antibacterial properties of zinc oxide nanoparticles (ZnO-NPs) have attracted a lot of attention from scientists all over the world, especially since nanotechnology has been used to create particles with dimensions in the nanometer range. Between hundreds of nanometers and tens of micrometers, there are several bacteria. Due to their increased specific surface area and decreased particle size, which increases particle surface reactivity, ZnO-NPs have appealing antibacterial capabilities (Sirelkhatim et al. 2015). Inorganic antimicrobial agents' non-specific action has drawn more attention to the usage of zinc oxide nanoparticles (ZnO NPs) to enhance the battle against microbial resistance. ZnO NPs' tiny particle size and large surface area can increase surface reactivity by enhancing antibacterial activity. Additionally, as the surface characteristics of nanomaterials modify their interactions with cells, this may prevent ZnO NPs from having their intended antimicrobial effect. Surface modifiers covering ZnO NPs can therefore play a role in moderating antimicrobial activity.(da Silva et al. 2019). Zinc oxide nanoparticles exhibit high catalytic efficiency, strong adsorption ability, high isoelectric point , biocompatibility, and fast electron transfer kinetics for biosensing purposes. They also have potential applications in a variety of fields, including optical, piezoelectric, magnetic, and gas sensing.(da Silva et al. 2019; Jamieson et al. 2007), (Kim 2007),("A Non-Aqueous Synthesis, Characterization of Zinc Oxide Nanoparticles and Their Interaction with DNA" 2009),("A Non-Aqueous Synthesis, Characterization of Zinc Oxide Nanoparticles and Their Interaction with DNA" 2009, "Effects of Native Defects on Optical and Electrical Properties of ZnO Prepared by Pulsed Laser Deposition" 2000),("A Novel Method for the Preparation of NH3 Sensors Based on ZnO-In Thin Films" 1995),("Nanostructures of Zinc Oxide" 2004).

S. Mutans is thought to be a key player in the development of dental caries in both humans and animals. S. Mutants prefer to colonize the surfaces of human teeth and prosthetics(Hamada and Slade 1980) . S. mutans was shown to be a major determinant in children's caries status, indicating that the relative amounts of these two bacteria in the oral cavity are crucial to the development of caries.(Ge et al. 2008). Although there are over 700 bacterial taxa in the oral cavity, they are not all found in every mouth. The composition differs in various areas of the oral cavity, with the dorsum of the tongue having a particularly high and diversified bacterial burden. Although the majority of these bacteria are benign, some of them have the potential to induce periodontal disease or oral diseases like caries . Streptococcus mutans and other oral streptococci have been linked to pyogenic and other infections in the mouth, heart, joints, skin, muscle, and central nervous system . (Forssten, Björklund, and Ouwehand 2010)

Our team has extensive knowledge and research experience that has translate into high quality publications (Neelakantan, Grotra, and Sharma 2013; Aldhuwayhi et al. 2021; Sheriff, Ahmed Hilal Sheriff, and Santhanam 2018; Markov et al. 2021; Jayaraj et al. 2015; Paramasivam et al. 2020; Li et al. 2020; Gan et al. 2019; Dua et al. 2019; Mohan and Jagannathan 2014)

AIM

The aim of this study is to assess the antibacterial activity of zinc oxide nanoparticles against streptococcus mutans using the extract of Picrorhiza kurroa

MATERIALS AND METHODS

A nutrient broth was prepared to conduct and assess minimum inhibitory concentrations

The prepared nutrient broth was sterilized and 6 ml was added in five separate test tubes

In all the five test tubes streptococcus mutans was added in the range of 5×10°CFU/ml

Picrorhiza kurroa extract is added in the first three test tubes at concentrations of 25, 50, and 100 mL, respectively.

In the other two test tubes one was taken to be standard with antibiotics and the other was taken to be positive control

The incubation was done under a suitable condition for varied time intervals (1st hour , 2nd hour , 3rd hour , 4th hour)

The percentage of dead cells is calculated at a wavelength of 600 nm at regular time intervals .

A graph was recorded with the concentration of the extract on the x-axis and the y-axis was taken as optical density and the time intervals were denoted by different colors

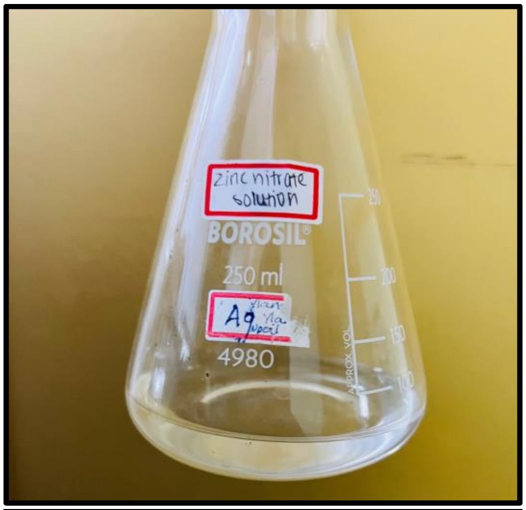


FIGURE 1: (Zinc Nitrate Solution)

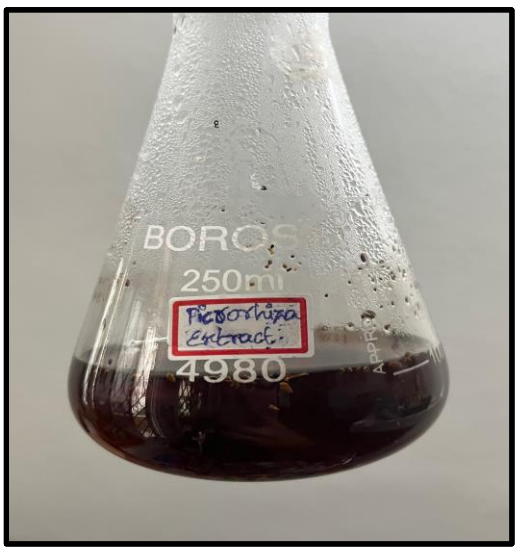


FIGURE 2: (Picrorhiza Extract)

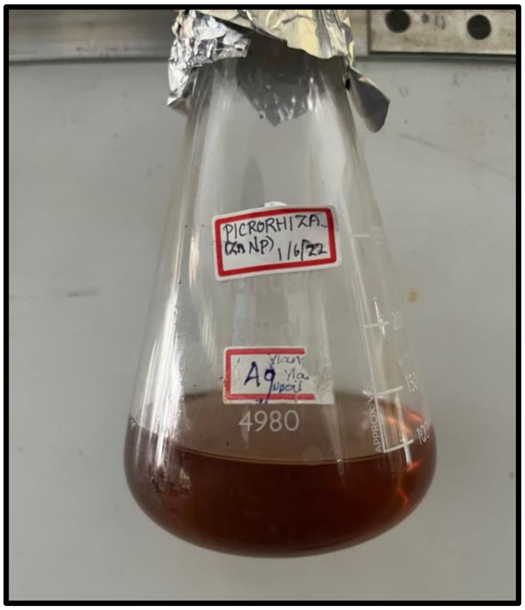
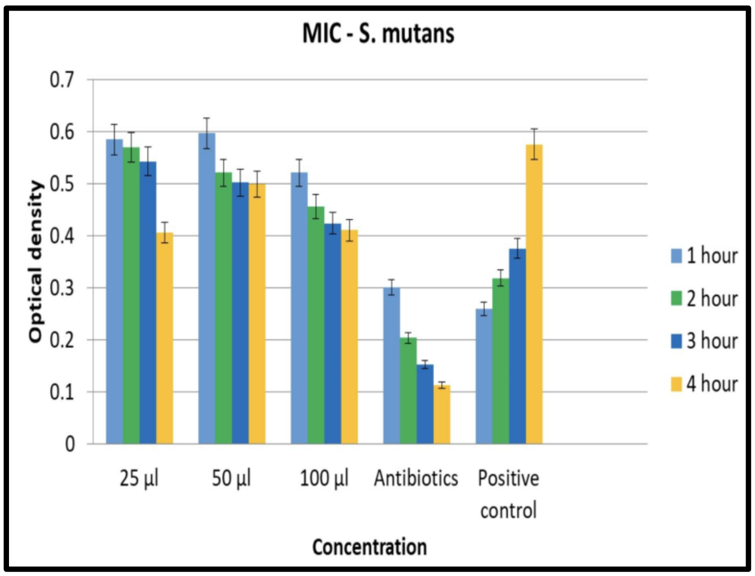


FIGURE 3: (Picrorhiza - Zn Np Extract) - Prepared Extract

RESULTS



GRAPH 1: represents minimal inhibitory concentration - S.mutans. X axis represents concentration in microlitre Y axis represents optical density.As the time increases inhibitory level decreases .25 microlitre concentration is more efficient.

DISCUSSION

In the MIC - S. mutans graph the x-axis designates the different concentrations of the extract (ie., 25µL, 50µL, 100µL) , the positive control with no extract and the commercially bought antibiotic with standard antibacterial activities . This is to compare the antibacterial activity of the extract to the standard antibiotic and positive control . The y-axis in this graph measures the optical density (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7)

Color indications have been given for each hour . The color light blue , green , dark blue , yellow depicts the 1st , 2nd , 3rd and 4th hour respectively . These color indications were given for simpler evaluation .

In the first test tube where the concentration of the extract was 25µL the optical density in the first four hours was noted to be 0.586 , 0.567, 0.553 and 0.409 correspondingly .

The second test tube has a concentration of 50µL of the extract . The optical density throughout four hours was noted to be 0.597 , 0.524, 0.507 and 0.5 respectively

Similarly the third test tube has a concentration of 100µL of the extract . The optical density throughout four hours was noted to be 0.526 , 0.467 , 0.428 and 0.417 respectively

The fourth test tube contains commercially available antibiotics . The optical density of these antibiotics was noted for four hours and it was noted to be 0.3, 0.207 , 0.161and 0.123 correspondingly . The final test tube contains only the bacteria . Major growth of the bacteria was noted.

The findings of a previous investigation demonstrated the bactericidal efficacy of composite resins including zinc oxide nanoparticles and silver nanoparticles against Streptococcus mutans and Lactobacillus, as the number of colonies of viable bacteria in the composite resin plates in the control group was much greater than that in the two other groups (Kasraei et al. 2014).

Some research was conducted with eight plant species traditionally used in South Africa to treat oral ailments had their ethanol extracts tested for in vitro antibacterial efficacy against oral infections. Using micro dilution to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol extracts against these microorganisms, it was demonstrated that, in the majority of cases, Gram negative bacteria were more resistant to the plant extracts than Gram positive bacteria.(Forssten, Björklund, and Ouwehand 2010; “Antimicrobial Activity of Medicinal Plants against Oral Microorganisms” 2008)

In comparison to HEEPk and bare CuO, the CuNPs-Pk exhibits excellent antibacterial activity against bacterial and fungal infections. Selected strains of Escherichia coli, Staphylococcus aureus, and Aspergillus niger were tested against various doses of hydroethanolic extract of Picrorhiza kurroa rhizomes. Dimethyl sulfoxide (DMSO) was utilised as a control for a few strains while ciprofloxacin and fluconazole were employed as standards. The particles were measured to have a diameter of 20–40 nm and a size of 275-285 nm. The presence of several functional groups was confirmed by FTIR analysis of biosynthetic nanoparticles (flavonoids, glycosides, tannins, phenols). Biosynthesized nanoparticles' spherical form and size (20–40 nm) have been predicted by SEM and TEM, and these particles have demonstrated excellent antibacterial activity against a number of pathogenic species, including Escherichia coli, Staphylococcus aureus, and Aspergillus niger than that of HEEpk and bare CuO . (Prakash et al. n.d.)

CONCLUSION

From the results of the MIC - S. mutans we can infer that on the usage of Picrorhiza kurroa-ZnO extract at the concentration of 25 microliters (at the fourth hour) and at 100 microliter the virulence of streptococcus mutans was decreased . To conclude that the Picrorhiza kurroa- ZnO extract possess significant antibacterial activity against streptococcus mutans

LIMITATIONS

Only one graph was laid out to test the antibacterial effect . This study could be developed further for more accurate results .

AUTHOR CONTRIBUTIONS

Ms. Katheeja Rilah: Literature search , survey , experimental data collection, analysis , manuscript writing

Dr.Rajesh Kumar - Research expert

Mrs. Sangeetha : Study design , data verification , manuscript drafting

CONFLICT OF INTERESTS

None to declare

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REFERENCES

1. "A Comprehensive Phytochemical, Ethnomedicinal, Pharmacological Ecology and Conservation Status of Picrorhiza Kurroa Royle Ex Benth.: An Endangered Himalayan Medicinal Plant." 2021. Process Biochemistry 109 (October): 72–86.
2. Aldhuwayhi, Sami, Sreekanth Kumar Mallineni, Srinivasulu Sakhamuri, Amar Ashok Thakare, Sahana Mallineni, Rishitha Sajja, Mallika Sethi, Venkatesh Nettam, and Azher Mohiuddin Mohammad. 2021. "Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey." Risk Management and Healthcare Policy 14 (July): 2851–61.
3. "A Non-Aqueous Synthesis, Characterization of Zinc Oxide Nanoparticles and Their Interaction with DNA." 2009. Synthetic Metals 159 (23-24): 2443–52.
4. "A Novel Method for the Preparation of NH3 Sensors Based on ZnO-In Thin Films." 1995. Sensors and Actuators. B, Chemical 25 (1-3): 588–90.
5. "Antimicrobial Activity of Medicinal Plants against Oral Microorganisms." 2008. Journal of Ethnopharmacology 119 (3): 473–77.
6. "Antioxidant and Antimicrobial Activity Displayed by a Fungal Endophyte Alternaria Alternata Isolated from Picrorhiza Kurroa from Garhwal Himalayas, India." 2021. Biocatalysis and Agricultural Biotechnology 33 (May): 101955.
7. "Antioxidant and Anti-Neoplastic Activities of Picrorhiza Kurroa Extracts." 2011. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association 49 (2): 363–69.
8. Dua, Kamal, Ridhima Wadhwa, Gautam Singhvi, Vamshikrishna Rapalli, Shakti Dhar Shukla, Madhur D. Shastri, Gaurav Gupta, et al. 2019. "The Potential of siRNA Based Drug Delivery in Respiratory Disorders: Recent Advances and Progress." Drug Development Research 80 (6): 714–30.
9. "Effects of Native Defects on Optical and Electrical Properties of ZnO Prepared by Pulsed Laser Deposition." 2000. Materials Science and Engineering B 71 (1-3): 301–5.

10. Forssten, Sofia D., Marika Björklund, and Arthur C. Ouwehand. 2010. “Streptococcus Mutans, Caries and Simulation Models.” Nutrients 2 (3): 290–98.
11. Gan, Hongyun, Yaqing Zhang, Qingyun Zhou, Lierui Zheng, Xiaofeng Xie, Vishnu Priya Veeraraghavan, and Surapaneni Krishna Mohan. 2019. “Zingerone Induced Caspase-Dependent Apoptosis in MCF-7 Cells and Prevents 7,12-Dimethylbenz(a)anthracene-Induced Mammary Carcinogenesis in Experimental Rats.” Journal of Biochemical and Molecular Toxicology 33 (10): e22387.
12. Ge, Y., P. W. Caufield, G. S. Fisch, and Y. Li. 2008. “Streptococcus Mutans and Streptococcus Sanguinis Colonization Correlated with Caries Experience in Children.” Creces / CONIN 42 (6): 444–48.
13. Hamada, S., and H. D. Slade. 1980. “Biology, Immunology, and Cariogenicity of Streptococcus Mutans.” Microbiological Reviews 44 (2): 331–84.
14. Jamieson, Timothy, Raheleh Bakhshi, Daniela Petrova, Rachael Pocock, Mo Imani, and Alexander M. Seifalian. 2007. “Biological Applications of Quantum Dots.” Biomaterials 28 (31): 4717–32.
15. Jayaraj, Gifrina, Pratibha Ramani, Herald J. Sherlin, Priya Premkumar, and N. Anuja. 2015. “Inter-Observer Agreement in Grading Oral Epithelial Dysplasia – A Systematic Review.” Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. https://doi.org/10.1016/j.ajoms.2014.01.006.
16. Jiang, Jinhuan, Jiang Pi, and Jiye Cai. 2018. “The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications.” Bioinorganic Chemistry and Applications 2018 (July). https://doi.org/10.1155/2018/1062562.
17. Kant, K., M. Walia, V. K. Agnihotri, Vijaylata Pathania, and B. Singh. 2013. “Evaluation of Antioxidant Activity of Picrorhiza Kurroa (Leaves) Extracts.” Indian Journal of Pharmaceutical Sciences 75 (3): 324–29.
18. Kasraei, Shahin, Lida Sami, Sareh Hendi, Mohammad-Yousef Alikhani, Loghman Rezaei-Soufi, and Zahra Khamverdi. 2014. “Antibacterial Properties of Composite Resins Incorporating Silver and Zinc Oxide Nanoparticles on Streptococcus Mutans and Lactobacillus.” Restorative Dentistry & Endodontics 39 (2): 109–14.
19. Kim, Kelly Y. 2007. “Nanotechnology Platforms and Physiological Challenges for Cancer Therapeutics.” Nanomedicine: Nanotechnology, Biology, and Medicine 3 (2): 103–10.
20. Li, Zhenjiang, Vishnu Priya Veeraraghavan, Surapaneni Krishna Mohan, Srinivasa Rao Bolla, Hariprasath Lakshmanan, Subramanian Kumaran, Wilson Aruni, et al. 2020. “Apoptotic Induction and Anti-Metastatic Activity of Eugenol Encapsulated Chitosan Nanopolymer on Rat Glioma C6 Cells via Alleviating the MMP Signaling Pathway.” Journal of Photochemistry and Photobiology B: Biology. https://doi.org/10.1016/j.jphotobiol.2019.111773
21. Markov, Alexander, Lakshmi Thangavelu, Surendar Aravindhan, Angelina Olegovna Zekiy, Mostafa Jarahian, Max Stanley Chartrand, Yashwant Pathak, Faroogh Marofi, Somayeh Shamlou, and Ali Hassanzadeh. 2021. “Mesenchymal Stem/stromal Cells as a Valuable Source for the Treatment of Immune-Mediated Disorders.” Stem Cell Research & Therapy 12 (1): 192.
22. Mohan, Meenakshi, and Nithya Jagannathan. 2014. “Oral Field Cancerization: An Update on Current Concepts.” Oncology Reviews 8 (1): 244.
23. “Nanostructures of Zinc Oxide.” 2004. Materials Today 7 (6): 26–33.
24. Neelakantan, Prasanna, Deeksha Grotra, and Subash Sharma. 2013. “Retreatability of 2 Mineral Trioxide Aggregate-Based Root Canal Sealers: A Cone-Beam Computed Tomography Analysis.” Journal of Endodontia 39 (7): 893–96.

25. Paramasivam, Arumugam, Jayaseelan Vijayashree Priyadharsini, Subramanian Raghunandhakumar, and Perumal Elumalai. 2020. "A Novel COVID-19 and Its Effects on Cardiovascular Disease." Hypertension Research: Official Journal of the Japanese Society of Hypertension.
26. "Phytochemical Screening and Antimicrobial Activity of Picrorrhiza Kurroa, an Indian Traditional Plant Used to Treat Chronic Diarrhea." 2016. Arabian Journal of Chemistry 9 (November): S1307–13.
27. "Picrorhiza Kurroa: A Promising Traditional Therapeutic Herb from Higher Altitude of Western Himalayas." 2020. Journal of Herbal Medicine 23 (October): 100358.
28. "'Picrosides' from Picrorhiza Kurroa as Potential Anti-Carcinogenic Agents." 2019. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie 109 (January): 1680–87.
29. Prakash, Vinit, Anjana Kumari, Harpreet Kaur, Manoj Kumar, Sumeet Gupta, and Ritu Bala. n.d. "Green Synthesis, Characterization and Antimicrobial Activities of Copper Nanoparticles from the Rhizomes Extract of Picrorhiza Kurroa." Pharmaceutical Nanotechnology 9 (4): 298–306. Accessed October 11, 2022.
30. Sheriff, K. Ahmed Hilal, K. Ahmed Hilal Sheriff, and Archana Santhanam. 2018. "Knowledge and Awareness towards Oral Biopsy among Students of Saveetha Dental College." Research Journal of Pharmacy and Technology. https://doi.org/10.5958/0974-360x.2018.00101.4.
31. Silva, Bruna Lallo da, Marina Paiva Abuçafy, Eloisa Berbel Manaia, João Augusto Oshiro Junior, Bruna Galdorfini Chiari-Andréo, Rosemeire C. L. R. Pietro, and Leila Aparecida Chiavacci. 2019. "Relationship Between Structure And Antimicrobial Activity Of Zinc Oxide Nanoparticles: An Overview." International Journal of Nanomedicine 14 (December): 9395–9410.
32. Sirelkhatim, Amna, Shahrom Mahmud, Azman Seeni, Noor Haida Mohamad Kaus, Ling Chuo Ann, Siti Khadijah Mohd Bakhori, Habsah Hasan, and Dasmawati Mohamad. 2015. "Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism." Nano-Micro Letters 7 (3): 219–42.