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Anticariogenic Activity Of Novel Herbal Formulations (Amla , Neem) Mediated Silver Nanoparticles - An In vitro study

Research Article

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Abstract

Aim: The aim of the study is to evaluate the anticariogenic activity of herbal formulations mediated silver nanoparticles against S. Aureus , C.Albicans , S. Mutans and E.. Faecalis .
Objective Of Study: 1) To prepare herbal formulations 2) To synthesize the silver nanoparticles using herbal formulations 3) To study the anticariogenic activity of silver nanoparticles
Materials And Methods: Azadirachta indica and Amla leaves were dried and powdered, which were made into herbal formulation. Silver nitrate (1 millimolar) was dissolved in 80 ml of distilled water, to that 20ml of filtered Amla, Neem plant extracts were added and kept in an orbital shaker for 2 days. To determine the antibacterial activity of silver nanoparticles Agar well diffusion method was done. Bacterial suspensions were dispersed with various organisms namely (S. Aureus , C.Albicans, S. Mutans and E.. Faecalis) on the surface of the agar plates containing Muller -Hinton agar . Readings were taken using U.V spectrophotometer at (250 -750 nm) and Centrifugation done (8000 rpm for 10 mins) Pellets were collected. Statistical analysis done using One way ANOVA and post Hoc test. P value was analysed.
Results: After the synthesis of silver nanoparticles color change was observed , the particles collected were then characterized and the peak value was seen at 425nm using UV- Spectroscopy. As the concentration of silver nanoparticles increased the zone of inhibition also increased in size.
Conclusion: Silver nanoparticles were synthesized using Amla, Neem herbal formulations. These formulations were effective against strains of S. Mutans at all concentrations and against S. Aureus at 100 microliter concentration and not effective against E. Faecalis.

Keywords: Green Synthesis ; Silver Nanoparticles; Neem Extracts; Phyllanthus Emblica.

Introduction

Azadirachta Indica (neem), Indian Lilac or margosa is one of the most well known subcontinent Indian native species, which is known for its bioactivity .Biogenic synthesis of the silver nanoparticles (AgNPs) using the plants has become one of the promising substitute to conventional chemical synthesis method. As the Silver nanoparticles have received high attention due to their extraordinary biological activities they are used in drug delivery, bio-labeling, sensing, food preservation, wound healings, water purifications and cosmetics[1, 2]. The use of extract from neem leaves has shown synergistic antibacterial effects .

As there are different methods for synthesizing silver nanoparticles , that includes chemical reduction , photochemical reduction vaporization , electrochemical reduction and thermal synthesis thus increasing its interest in developing to produce AgNPs using green synthesis and plant extracts . Several studies have reported that the silver nanoparticles which were made up of different noble metals like Ag, Cu, and Au, which can be applied to kill both resistant and non resistant bacteria [3,4]. The plant mediated green synthesis of AgNps are developing into a new branch of

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nanotechnology. In this way it has been less toxic and hazardous materials and environmentally benign solvents,[5] simple, rapid and cost effective,[2]consumes less energy and performs under moderate operation conditions,[6] combines the potency of both silver nanoparticles and plant active ingredients.

Plant-mediated nanoparticle synthesis, which is also commonly known as "green synthesis", as is the most widely acknowledged way of synthesis of silver nanoparticles because of the diversified cellular metabolites present in plant extracts. Plant mediated synthesis of silver nanoparticles is most preferable over microbe-mediated synthesis. The latter is not feasible and requires high aseptic conditions, time taking process and long incubation periods.[7] The biodiversity of plants along with their phytochemical variations is the most accredited factor influencing the physical, chemical, and biological properties of biosynthesized nanoparticles as they generally get fabricated with nanoparticles during the synthesis progression.[8]

Recent research reported that AgNPs have been synthesized using a variety of natural plants from the fruit extract of Emblica officinalis[9], leaves extract of Citrus limon (Vankar and Shukla, 2012), green tea (Camellia sinensis)[10], Coffea Arabica[11], neem (Azadirachta indica)[12], Acalypha indica (Krishnaraj et al., 2012), Aloe vera plant extract[13], latex of Jatropha gossypifolia[14], root extract of Morinda citrifolia[15] etc.

In the synthesis of silver nanoparticles, plant extracts and microbes act as the reducing agents for reducing $Ag+$ to $Ag0$ and capping or stabilizing agents for preventing the aggregation of the nanoparticles.[15] Various mechanisms attributed to the antimicrobial activity shown by AgNPs, however, the exact mechanism is yet to be elucidated because the nanoparticles act on different organisms in different ways. The development of antibiotic resistance by bacterial cells upon a frequent use of antibiotics is a serious concern [16]. Recently, the promising antimicrobial potential of AgNPs against both gram-positive and gram-negative bacterial cells and their stability has attracted scientific interest. Furthermore, by the use of AgNPs, the bacterial cells are less prone to develop antibacterial resistance [17].

Aloe vera leaves which have been used as medicinal plants that possess anti-inflammatory activity, promote wound and burn-healing, UV protection, antiarthritic properties and have been noted for their antibacterial properties[16-19]. There are numerous available biologically active constituents of aloe vera leaves. These include lignin, hemicellulose, pectins which can be used in the reduction of silver ions [22]. It has been believed that large enzymes and proteins in aloe vera extract have been weakly bound to silver ions and help in the function as a complexing agent. Azadirachta indica (commonly known as neem) which is a member of the Meliaceae family used for the synthesis of silver nanoparticles. This is a medicinal plant and is used for the treatment of bacterial, fungal, viral, and many types of skin ailments since ancient times. The aqueous neem extract which is used for synthesizing various nanoparticles such as gold, zinc oxide, silver, etc. Terpenoids and flavanones are the two important phytochemicals present in the neem which play a vital role in stabilizing the nanoparticle and also act as capping and reducing agents [23]. Aqueous neem leaf extract reduces silver salt to silver nitrate, this capped nanoparticle with neem extract exhibit antibacterial activity.[24]

In the present study, the green synthesized silver nano-particle and antibacterial effect as its role in caries activity was studied by analysing the bacterial count using the disc diffusion method. Pertaining to this study the concentrations of silver nanoparticles was effective against the growth of gram-positive and gram -negative bacteria .Previously our team has a rich experience in working on various research projects across multiple disciplines [20-34] Now the growing trend in this area motivated us to pursue this project.

Materials And Methods

It was an in vitro study conducted in the month of November 2020 – December 2020 in the city of Chennai, Tamil Nadu.

Plant Material And Characterisation Of The Silver Particles

Leaves of Phyllanthus emblica and Azadirachta indica were collected from University Campus in the month of November from chennai, Tamil Nadu, India. Leaves of Phyllanthus emblica and Azadirachta indica were thoroughly washed under running water to remove the dirt and dust on the surface of the leaves. The leaves were air dried for 10 days and then kept in the hot air oven at 600C for 24-48 hours. These leaves were then grounded to a fine powder. 1 mM silver nitrate was dissolved in 80 ml of distilled water , to that 20 ml of filtered Amla , Neem plant extracts were added and kept in an orbital shaker for 2 days . Readings were taken using a U.V Spectrophotometer (250 - 750 nm) . The supernatants were heated at 50 to 950C. A change in the colour of the solution was observed during the heating process within 10-15 minutes. The colour changes indicate the formation of silver nanoparticles (SNPs).The reduction of pure $Ag2+$ ions were monitored by measuring the UV spectrum of the reduction media at 5 hours after diluting a small sample in distilled water by using systronic 118 UV Spectrophotometer as standardised in the study by Savithramma et al[35]

Antimicrobial Analysis

The antibacterial activity was assessed using a disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification, bacterial cultures of S. Aureus , C.Albicans , S. Mutants and E.. Faecalis were swabbed on the agar plates. The sterile discs were then dipped in silver nanoparticles solution (10 mg/ml) and placed in the nutrient agar plate and kept for incubation at 370C for 24 hours. Zones of inhibition were measured. The experiments were repeated thrice and mean values of zone diameter were presented.[36]

Antifungal Activity

The dextrose agar plates were then prepared, sterilized and solidified. After solidification fungal cultures were also swabbed on these plates. Then sterile discs were then dipped in the silver nanoparticles solution (10mg/ml) and placed in the agar plate and kept for incubation for 7 days. The zone of inhibition was measured after 7 days .

Statistical Analyses

The collected data was tabulated into Microsoft office Excel 2013 transferred to SPSS version 26.0 software (SPSS, Chicago, IL, USA) for statistical analysis. Descriptive data analysis was done to find the mean and standard deviation for the data. This data was analyzed statistically using the chi square test to see if the results were statistically significant. The confidence interval was set at 95%. Data are represented as the average values with standard error of at least three values of each independent experiment.

Result

As shown in Table 1, a minimum of 10mm zone of inhibition was observed for both C.albicansand S.mutans, whereas E. faecalis showed a minimum of 22mm and S.aureusshowed only 9mm in diameter. Study appeared to have higher results for C.albicans as compared to the antibiotic group at all concentrations and at higher concentrations for S. aureus. For E. faecalis and S. mutansthe formulations appeared to be half as effective as the antibiotic used. These preliminary data indicated that AgNPs, as well as Ag+,have antibacterial activity.

Discussion

Biologically synthesized AgNPs have been reported to be promising therapeutic molecules with significant antimicrobial and antiviral activities[37]. These are environmentally friendly processes that avoid the toxicity of chemicals. Compared to toxicity of chemicals, plant extracts contain functional substances, including cyclic peptides, sorbic acid, citric acid, euphol, polyhydroxy limonoids, ascorbic acid, retinoic acid, tannins, ellagic acid, and gallic acid, among others, are strongly believed to play a crucial role in the bioreduction and stabilization of nanoparticles[38]. Overall, this study reported green-synthesized AgNPs mediated by Azadirachta indica and Aloe barbadensis plant extracts and demonstrated good antibacterial activity.

Biosynthesis of nanoparticles employing the use of plant extract

Figure 1 :The Bar chart representing a zone of inhibition was observed for both C.albicans and S.mutans was 10mm , whereas E. faecalis showed a minimum of 22mm followed by S.aureus showed only 9mm in diameter. Study appeared to have higher results for C.albicans as compared to the antibiotic group at all concentrations and at higher concentrations for S. aureus. For E. faecalis and S. mutans the formulations appeared to be half as effective as the antibiotic used.

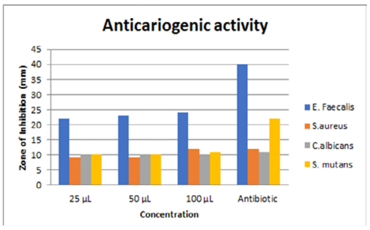


Figure 2 :The figure 2 represents the Zone of inhibition against E. faecalis with varying concentrations of (Amla , Neem) silver nanoparticles .

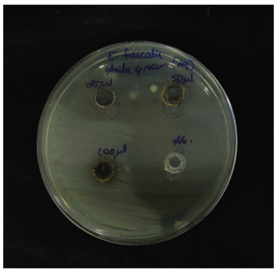


Figure 3 :The figure 2 represents the Zone of inhibition against S.Aureus with varying concentrations of (Amla , Neem) silver nanoparticles .

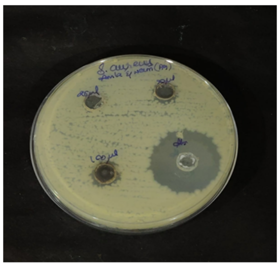
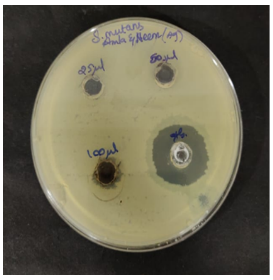


Figure 4 :The figure 2 represents the Zone of inhibition against C.Albicans with varying concentrations of (Amla , Neem) silver nanoparticles .

Figure 5 :The figure 2 represents the Zone of inhibition against S. Mutans with varying concentrations of (Amla , Neem) silver nanoparticles .



is a noble strategy for biosynthesis reaction because of their non-toxic properties and thus provide natural capping agents[39,40]. One of the probable mechanisms is that silver nanoparticles attach to the surface of the cell membrane, the respiratory function and permeability of the bacterial cells become unstable[41]. It was speculated that the hydroquinones in the aloe vera plant extract act as the reducing agents. Reactive Oxygen species (ROS) such as singlet oxygen O2, hydroxyl radical OH− and peroxide radical O2−, are produced by silver nanoparticles which are toxic to the bacteria[42]. It has been noted that the susceptibility of different types of bacteria was attributed to the structure of their bacterial cell walls.

We have investigated the zone of inhibition of AgNPs as compared against the reference antibiotics drug . This study clearly indicates that the stabilized AgNPs has excellent antimicrobial activity against gram positive organisms of S. aureus and C.albicans . In the case of C.albicans, the result shows that the antibacterial activity at 25 μL of the AgNPs is equal to antibacterial activity at 100 μL . For the case of S. aureus, the result shows an equal of 100% of the antibacterial activity of the antibiotic at 100 μL as compared to 25 μL. For E.faecalis and S.mutans the formulations appeared to be either half or slightly less effective as the reference antibiotic used even at 100 μL concentrations. Previous studies indicated that the silver ion released from AgNPs was responsible for antibacterial activity[19]. The free silver ion can then bind with the thiol groups of enzymes[43]. In a previous study[44], the antimicrobial activity of silver nanoparticles for Gram-positive bacteria was less compared to Gram-negative bacteria. Similar results have been reported earlier for neem as well as other plant extracts. This is attributed to the peptidoglycan layer which is negatively charged and prevents the free entry of Ag ions into the cell wall[45,46].

The present work supports the medicinal values of these plants also reveals a simple, rapid and economical route to synthesis of silver nanoparticles; and their capability of rendering the antimicrobial efficacy. The synthesized Silver nanoparticles enhance the therapeutic efficacy and strengthen the medicinal values of these plants.

Our institution is passionate about high quality evidence based research and has excelled in various fields [47-57]

Conclusion

Biosynthesis of silver nanoparticles using Amla , Neem herbal formulations were done. They were effective against the strains of S.Mutansat all concentrations and followed by S.Aureusat 100 microliter concentration and it has been found that it was not effective against E.Faecalis .

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References

[1]. Masum MMI, Siddiqa MM, Ali KA, Zhang Y, Abdallah Y, Ibrahim E, et al. Biogenic Synthesis of Silver Nanoparticles Using Phyllanthus emblica Fruit Extract and Its Inhibitory Action Against the Pathogen Acidovorax oryzae Strain RS-2 of Rice Bacterial Brown Stripe. Front Microbiol. 2019 Apr 26;10:820. Pubmed PMID: 31110495.
[2]. Zhang S, Tang Y, Vlahovic B. A Review on Preparation and Applications of Silver-Containing Nanofibers. Nanoscale Res Lett. 2016 Dec;11(1):80. Pubmed PMID: 26858162.
[3]. Escárcega-González CE, Garza-Cervantes JA, Vázquez-Rodríguez A, Montelongo-Peralta LZ, Treviño-González MT, Díaz Barriga Castro E, et al. In vivo antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using Acacia rigidula as a reducing and capping agent. Int J Nanomedicine. 2018 Apr 17;13:2349-2363. Pubmed PMID: 29713166.
[4]. Bindhu MR, Umadevi M. Antibacterial activities of green synthesized gold nanoparticles. Materials Letters. 2014 Apr 1;120:122-5.
[5]. Kerker M. The optics of colloidal silver: something old and something new. Journal of Colloid and Interface Science. 1985 Jun 1;105(2):297-314.
[6]. Handoko CT, Huda A, Bustan MD, Yudono B, Gulo F. Green synthesis of silver nanoparticle and its antibacterial activity. Rasayan J. Chem. 2017;10(4):1137.
[7]. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research. 2011 Jul;13(7):2981-8.
[8]. Sharma G, Nam JS, Sharma AR, Lee SS. Antimicrobial Potential of Silver Nanoparticles Synthesized Using Medicinal Herb Coptidis rhizome. Molecules. 2018 Sep 5;23(9):2268. Pubmed PMID: 30189672.
[9]. Raj R K, D E, S R. β-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. J Biomed Mater Res A. 2020 Sep;108(9):1899-1908. Pubmed PMID: 32319188.
[10]. Nakhjavani M, Nikkhah V, Sarafraz MM, Shoja S, Sarafraz M. Green synthesis of silver nanoparticles using green tea leaves: Experimental study on the morphological, rheological and antibacterial behaviour. Heat and Mass Transfer. 2017 Oct;53(10):3201-9.
[11]. Dhand V, Soumya L, Bharadwaj S, Chakra S, Bhatt D, Sreedhar B. Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Mater Sci Eng C Mater Biol Appl. 2016 Jan 1;58:36-43. Pubmed PMID: 26478284.
[12]. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of radiation research and applied sciences. 2016 Jan 1;9(1):1-7.
[13]. Tippayawat P, Phromviyo N, Boueroy P, Chompoosor A. Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. PeerJ. 2016 Oct 19;4:e2589. Pubmed PMID: 27781173.
[14]. Borase HP, Patil CD, Salunkhe RB, Suryawanshi RK, Salunke BK, Patil SV. Transformation of aromatic dyes using green synthesized silver nanoparti-

cles. Bioprocess Biosyst Eng. 2014 Aug;37(8):1695-705. Pubmed PMID: 24525834.
[15]. Suman TY, Radhika Rajasree SR, Kanchana A, Elizabeth SB. Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using Morinda citrifolia root extract. Colloids Surf B Biointerfaces. 2013 Jun 1;106:74-8. Pubmed PMID: 23434694.
[16]. Vázquez B, Avila G, Segura D, Escalante B. Antiinflammatory activity of extracts from Aloe vera gel. J Ethnopharmacol. 1996 Dec;55(1):69-75. Pubmed PMID: 9121170.
[17]. Reynolds T, Dweck AC. Aloe vera leaf gel: a review update. J Ethnopharmacol. 1999 Dec 15;68(1-3):3-37. Pubmed PMID: 10624859.
[18]. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol Prog. 2006 Mar-Apr;22(2):577-83. Pubmed PMID: 16599579.
[19]. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res. 2000 Dec 15;52(4):662-8. Pubmed PMID: 11033548.
[20]. Govindaraju L, Gurunathan D. Effectiveness of Chewable Tooth Brush in Children-A Prospective Clinical Study. J Clin Diagn Res. 2017 Mar;11(3):ZC31-ZC34. Pubmed PMID: 28511505.
[21]. Christabel A, Anantanarayanan P, Subash P, Soh CL, Ramanathan M, Muthusekhar MR, et al. Comparison of pterygomaxillary dysjunction with tuberosity separation in isolated Le Fort I osteotomies: a prospective, multi-centre, triple-blind, randomized controlled trial. Int J Oral Maxillofac Surg. 2016 Feb;45(2):180-5. Pubmed PMID: 26338075.
[22]. Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery--a systematic review. Int J Oral Maxillofac Surg. 2013 Aug;42(8):974-80. Pubmed PMID: 23702370.
[23]. Mehta M, Deeksha, Tewari D, Gupta G, Awasthi R, Singh H, et al. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206-215. Pubmed PMID: 31136735.
[24]. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115-121. Pubmed PMID: 30451321.
[25]. Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, et al. The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol. 2014 Jan;13(1):44-58. Pubmed PMID: 24291220.
[26]. Kumar S, Sneha S. Knowledge and awareness regarding antibiotic prophylaxis for infective endocarditis among undergraduate dental students. Asian Journal of Pharmaceutical and Clinical Research. 2016;154.
[27]. Christabel SL, Gurunathan D. Prevalence of type of frenal attachment and morphology of frenum in children, Chennai, Tamil Nadu. World J Dent. 2015 Oct;6(4):203-7.
[28]. Kumar S, Rahman RE. Knowledge, awareness, and practices regarding biomedical waste management among undergraduate dental students. Asian Journal of Pharmaceutical and Clinical Research. 2017;10(8):341.
[29]. Sridharan G, Ramani P, Patankar S. Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Cancer Res Ther. 2017 Jul-Sep;13(3):556-561. Pubmed PMID: 28862226.
[30]. Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S. Herbs as an antioxidant arsenal for periodontal diseases. J Intercult Ethnopharmacol. 2016 Jan 27;5(1):92-6. Pubmed PMID: 27069730.
[31]. Thamaraiselvan M, Elavarasu S, Thangakumaran S, Gadagi JS, Arthie T. Comparative clinical evaluation of coronally advanced flap with or without platelet rich fibrin membrane in the treatment of isolated gingival recession. J Indian Soc Periodontol. 2015 Jan-Feb;19(1):66-71. Pubmed PMID: 25810596.
[32]. Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthuswami M, et al. Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations. PLoS One. 2016 Jun 9;11(6):e0156582. Pubmed PMID: 27280700.
[33]. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods. 2019 May;29(4):276-290. Pubmed PMID: 30461321.
[34]. Ramakrishnan M, Bhurki M. Fluoride, Fluoridated Toothpaste Efficacy And Its Safety In Children-Review. International Journal of Pharmaceutical Research. 2018 Oct 1;10(04):109-14.
[35]. Savithramma N, Rao ML, Rukmini K, Devi PS. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of ChemTech Research. 2011 Jan 1;3(3):1394-402.
[36]. Cruickshank R, editor. Medical microbiology: a guide to the laboratory diagnosis and control of infection. Livingstone; 1969.
[37]. Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM. A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. Nanoscale Research Letters. 2008 Apr;3(4):129-33.
[38]. Rajan R, Chandran K, Harper SL, Yun SI, Kalaichelvan PT. Plant extract synthesized silver nanoparticles: an ongoing source of novel biocompatible materials. Industrial Crops and Products. 2015 Aug 1;70:356-73.
[39]. harma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci. 2009 Jan 30;145(1-2):83-96. Pubmed PMID: 18945421.
[40]. Rai M, Ingle A. Role of nanotechnology in agriculture with special reference to management of insect pests. Appl Microbiol Biotechnol. 2012 Apr;94(2):287-93. Pubmed PMID: 22388570.
[41]. Kvítek L, Panáček A, Soukupova J, Kolář M, Večeřová R, Prucek R, et al. Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). The Journal of Physical Chemistry C. 2008 Apr 17;112(15):5825-34.
[42]. Carlson C, Hussain SM, Schrand AM, Braydich-Stolle LK, Hess KL, Jones RL, et al. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J Phys Chem B. 2008 Oct 30;112(43):13608-19. Pubmed PMID: 18831567.
[43]. Zhang Y, Cheng X, Zhang Y, Xue X, Fu Y. Biosynthesis of silver nanoparticles at room temperature using aqueous aloe leaf extract and antibacterial properties. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2013 Apr 20;423:63-8.
[44]. Roy P, Das B, Mohanty A, Mohapatra S. Green synthesis of silver nanoparticles using Azadirachta indica leaf extract and its antimicrobial study. Applied Nanoscience. 2017 Nov;7(8):843-50.
[45]. Ankanna ST, Prasad TN, Elumalai EK, Savithramma N. Production of biogenic silver nanoparticles using Boswellia ovalifoliolata stem bark. Digest Journal of Nanomaterials and Biostructures. 2010 Jun 1;5(2):369-72.
[46]. Kim SH, Lee HS, Ryu DS, Choi SJ, Lee DS. Antibacterial activity of silver-nanoparticles against Staphylococcus aureus and Escherichia coli. Microbiology and Biotechnology Letters. 2011;39(1):77-85.
[47]. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441-1448. Pubmed PMID: 31257588.
[48]. J PC, Marimuthu T, C K, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin Implant Dent Relat Res. 2018 Aug;20(4):531-534. Pubmed PMID: 29624863.
[49]. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241-1248. Pubmed PMID: 30044495.
[50]. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig. 2019 Sep;23(9):3543-3550. Pubmed PMID: 30552590.
[51]. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019 Apr;48(4):299-306. Pubmed PMID: 30714209.
[52]. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115-121. Pubmed PMID: 30451321.
[53]. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial. Clin Oral Investig. 2020 Sep;24(9):3275-3280. Pubmed PMID: 31955271.
[54]. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285-286. Pubmed PMID: 32416620.
[55]. R H, Ramani P, Ramanathan A, R JM, S G, Ramasubramanian A, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020 Sep;130(3):306-312. Pubmed PMID: 32773350.
[56]. Chandrasekar R, Chandrasekhar S, Sundari KKS, Ravi P. Development and validation of a formula for objective assessment of cervical vertebral bone age. Prog Orthod. 2020 Oct 12;21(1):38. Pubmed PMID: 33043408.
[57]. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Arch Oral Biol. 2018 Oct;94:93-98. Pubmed PMID: 30015217.