RESEARCH
O&G Forum 2024; 34 – 2s: 156 - 159

EVALUATING THE ANTIMICROBIAL EFFICACY AND CYTOTOXICITY OF BIOACTIVE COMPOUND BASED NANOPARTICLES AS A ORAL RINSE: A COMPREHENSIVE STUDY

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Abstract
Introduction: Research on nanotechnology has increased significantly in recent years, particularly in the areas of green synthesis and nanoparticle characterization. Pomegranate (*Punica granatum*) has been employed in several medical systems for its medicinal benefits, and one of its biological features has been identified as antibacterial activity. Green synthesis of AgNPs is shown to help in the formulation of potent antibacterial drugs for use against microorganisms that pose a threat to public health. This study aims to synthesize, characterize and assess the cytotoxic activity of *Punica granatum* mediated silver nanoparticles mouthrinse.
Materials and methods: 1 mm of Silver nitrate solution was prepared by dissolving 1 gram of silver nitrate powder in 90 ml of distilled water and is incorporated in 10 ml of PG extract. The combined solution was placed in magnetic orbital shaker for 24 hours. The solution was then centrifuged to obtain nanoparticle pellets. A mouthwash was prepared using the nanoparticles and its antimicrobial and antiplaque potential was assessed.
Results and Discussion: The Ag-PG nanoparticles showed a color change and its formation was further confirmed by UV-Visible spectroscopy which peaked at 350 nm. Different concentrations of nano-particles exhibited different levels of antimicrobial and anti plaque potential.
Conclusion: Thus, it can be concluded that the Punica granatum mediated silver nanoparticles based mouthwash that was formulated showed various spectrum of antimicrobial and anti plaque potential activity at different concentrations of nano-particles and is considered safe for use as a mouth rinse.
Keyword: Pomegranate, Mouthwash, Herbal Mouthwash, antimicrobial activity, Chlorhexidine alternatives, Silver Nanoparticles, Bioactive compound.

INTRODUCTION

Research on nanotechnology has increased significantly in recent years, particularly in the areas of green synthesis and nanoparticle characterization. This is because nanoparticles with a size of less than 100 nm are the most effective delivery systems for drugs and have numerous other biomedical uses (1). Nano-biotechnology, drug formulation and processing, physical methods,drug engineering, nano-crystallines, and nano-biomaterials are just a few of the fields that benefit from the creation of nanoparticles. There are now three ways to make nanoparticles: chemically, physically, and in a "green" way that uses plant based reducing agents such as microbe derived filtrates and concentrated plant extracts(2). These techniques are frequently expensive and produce harmful byproducts, but the green nanosynthesis technique has gained recognition as a low-cost and environmentally beneficial option(3). Plant components, such as proteins, enzymes, and carbohydrates, are employed to create nanoparticles that are easily able to interact with target biomolecules in the green production of NPs(4). Green synthesis of AgNPs can aid in the formulation of potent antibacterial drugs for use against microorganisms that pose a threat to public health(5). Recently, it has been discovered that manufactured AgNPs and the antibiotic levofloxacin can work in synergy to boost overall antibacterial activity. Numerous studies have revealed that manufactured AgNPs have lethal effects on many malignant and healthy cell lines, as well as well-known antibacterial activities against Gram-positive and Gram-negative infections. In addition, compared to silver ions alone, AgNPs are very effective because of a high surface-to-volume ratio, are able to quickly disrupt, and can infiltrate bacterial cells(6).

Pomegranate (*Punica granatum*) has been employed in several medical systems for its medicinal benefits, and one of its biological features has been identified as antibacterial activity.

Punica granatum (PG), one of the two species that make up the Punicaceae family, is the most common member(7). It serves as an antiparasitic agent and is regarded as "a pharmacy unto itself." Pomegranates have a long history of use in several branches of conventional medicine, sometimes in combination with other ingredients to treat ailments like lethargy, diarrhea, and ulcers(8). Pomegranates have a wide range of possible medicinal benefits, including the management and prevention of cancer, cardiovascular pathologies, diabetes Mellitus, dental diseases and UV radiation protection. Other potential uses include the treatment of obesity, arthritis, Alzheimer's disease, male infertility, and infant brain ischemia(9). In addition, pomegranate juice has been utilized for its antibacterial capabilities against Bacillus subtilis and E. coli, as well as for its anti-inflammatory effects to treat dental pathologies, microbial infections, antimicrobial resistance, gastrointestinal pathologies and intestinal parasites. *Punica* extract can be used as an anthelmintic agent, particularly in the treatment of various intestinal worms and vaginal infections. Additionally, *Punica granatum* cortex extract has shown bacteriostatic activity on several microbes isolated from the human stomach and intestine, and there is evidence of the existence of antimicrobial phenolic chemicals(10).

Hence, this study aims to synthesize, characterize and assess the antimicrobial activity of *Punica granatum* mediated silver nanoparticles mouthrinse.

MATERIALS AND METHOD

Formulation of *Punica granatum (PG)* extract:

10 grams of Punica granatum seeds were collected and washed. The seeds were then crushed using mortar and pestle finely, until a slurry was obtained. The slurry was then filtered using cotton mesh and filter papers to obtain the PG extract.

Synthesis of PG based nanoparticles:

1 mM of Silver nitrate solution was prepared by dissolving 1 gram of silver nitrate powder in 90 ml of distilled water. This solution was incorporated into 10 ml of PG extract. The combined solution was placed in a magnetic orbital shaker for 24 hours. The solution was then centrifuged at 8000 rpm for 10 minutes to obtain nanoparticle pellets. The pellets were then collected, washed and dried for further use.

Synthesis of PG based silver nanoparticles mouthwash:

A mouthwash was prepared using 0.01 g of Sodium Lauryl sulfate, 0.3 g sucrose, 0.001 g Sodium Benzoate in 8 ml of distilled water. Sodium lauryl sulfate acts as a foaming agent, sucrose as a sweetening agent and sodium benzoate as a preservative. 2 ml of the prepared PG nanoparticles were added to this mouthwash to make 10 ml of Ag-PG mouthwash.

Evaluation of Cytotoxicity by Brine Shrimp Lethality Assay:

Freshly hatched small brine shrimps (*Artemia salina*) larvae (Nauplii) were introduced into 5 wells. The prepared Ag-PG mouthwash was introduced into each of the wells in varying concentrations of the mouthwash- 5, 10, 15, 20 and 25 µL. One well was prepared as the control sample, in which mouthwash was not added. The samples were incubated without disturbances for 24 hours. The total number of live nauplii after the incubation period was noted and the data was graphically represented.

RESULTS AND DISCUSSION

The Ag-PG nanoparticles showed a color change and its formation was further confirmed by UV-Visible spectroscopy which showed a peak at 350 nm. Using the Brine Shrimp Lethality Test, the cytotoxic activity of Ag-PG mouthwash was evaluated. Due to the fact that all of the brine shrimp in the six wells where the extract was applied survived, the synthesized nanoparticles did not exhibit cytotoxic effect on brine shrimp. According to the results of the brine shrimp lethality test, on the first day, all nauplius survived, and on the second day, all nauplius survived. The toxicity remained the same regardless of the nanoparticle concentration, which had no effect on the nauplius.

Previous studies by Fernandes et al., 2018, tried preparing silver nanoparticles using pomegranate peel extract. They assessed the antimicrobial activity and the cytotoxic effect of the nanoparticles. They concluded that green-AgNP had significantly lower cytotoxicity than the corresponding chemical controls. Pomegranate peel extract generated stable AgNP with low cytotoxicity and antibacterial activity, encouraging its use in the biomedical industry(11).

Previous study conducted by Sparabombe et al., in 2019 studied the treatment of periodontitis using an all natural mouthwash. They tested the clinical efficacy in a single blind Randomized control trial. They concluded that the gingival inflammation and plaque buildup significantly reduced when the poly herbal mouthwash formula was used for three months continuously . It is noteworthy that neither group at any given time reported or saw any negative events or side effects(12). Numerous research on the use of natural extracts and plant products in the treatment of oral illnesses provide proof of their efficacy. It has been demonstrated that alternative herbal mouthwashes are more efficient than a conventional mouthwash in treating periodontal diseases in susceptible individuals. Yet, the long term efficacy is still debatable as the studies failed to do a long term follow up and follow a standardized methodology. Three months of testing against a placebo mouthwash to determine the effects of three herbal extracts (*Juniperus communis*, *Urtica dioica*, and *Achillea millefolium*) on plaque and gingivitis revealed no positive results(13).

Previous studies conducted by Umar et al in 2016 studied the antimicrobial effect of pomegranate mouthrinse on *Streptococcus mutans* count. They reported that Pomegranate mouthwash reduced the *S.mutans* colony count 10 minutes after administration of mouthwash. Pomegranate based mouthwash may prevent dental plaque formation by obstructing the actions of the microbes that generate plaque when used often in conjunction with toothpaste that has been strengthened with bioactive plant extracts(14). Additionally, the anti-inflammatory characteristics of pomegranate polyphenols may aid in soothing irritated tissues. The capacity of these germs to cling to the surface of the tooth is suppressed by pomegranate extract(15). In research from Ohio State, participants who rinsed with pomegranate solution had lower levels of total protein in their saliva, which is often higher in gingivitis patients and may be related to bacteria that create plaque. Rinsing with pomegranate juice also decreased the activity of the sucrose-degrading enzyme alpha-glucosidase in the saliva while increasing the activity of the antioxidant enzyme ceruloplasmin. Pomegranate may therefore also have an anticariogenic effect, which may be used to protect individuals from acquiring dental caries(16).

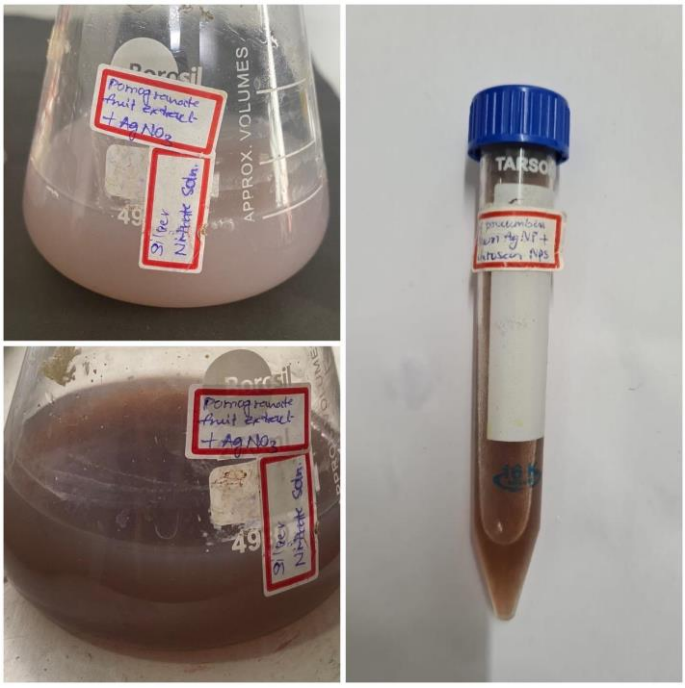

Figure 1: Images showing the green synthesis of the *Punica granatum* mediated silver nanoparticles.

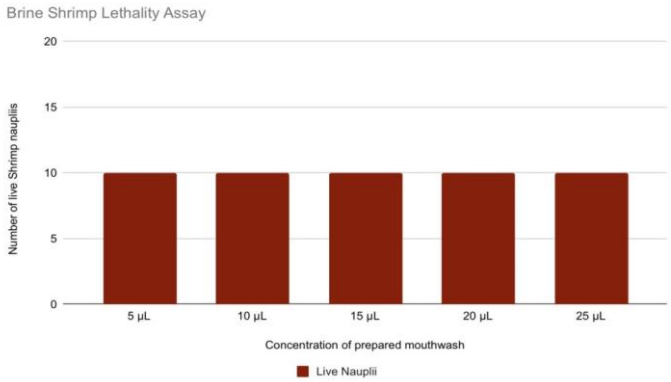

Figure 2: Bar graph depicting the Cytotoxic activity exhibited by Ag-PG mouthwash using brine shrimp lethality test. X- Axis shows concentration of the *Punica granatum* mediated silver nanoparticles mouthwash (µL) and Y-Axis shows number of live nauplii.

CONCLUSION

Within the limits of the study, it can be concluded that the Punica granatum mediated silver nanoparticles based mouthwash that was formulated showed the antimicrobial activities may be increased if active elements are refined and adequate dosage determined for correct administration. The use of herbs in dentistry ought to be based on proof of effectiveness and safety. With further analysis, there positively can be a niche for seasoning intracanal treatments in dentistry.Therefore in this study, Punica granatum with silver nanoparticles shows best inhibitory characteristic of Enterococcus faecalis and has no cytotoxic activity, and can be considered safe for use.

ACKNOWLEDGEMENT

We would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, and Saveetha University for providing us with support to conduct the study.

References

1. *Devanesan S, AlSalhi MS, Balaji RV, Ranjitsingh AJA, Ahamed A, Alfuraydi AA, et al. Antimicrobial and Cytotoxicity Effects of Synthesized Silver Nanoparticles from Punica granatum Peel Extract. Nanoscale Res Lett. 2018 Oct 4;13(1):315.*
2. *Abd-Elsalam KA. Green Synthesis of Silver Nanomaterials. Elsevier; 2021. 795 p.*
3. *Mares M, Lim SHE, Lai KS, Cristina RT. Antimicrobial Resistance: A One Health Perspective. BoD – Books on Demand; 2021. 210 p.*
4. *Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine. 2012 Jan;8(1):37–45.*
5. *He G, Qiao M, Li W, Lu Y, Zhao T, Zou R, et al. S, N-Co-Doped Graphene-Nickel Cobalt Sulfide Aerogel: Improved Energy Storage and Electrocatalytic Performance [Internet]. Vol. 4, Advanced Science. 2017. p. 1600214. Available from: http://dx.doi.org/10.1002/advs.201600214*
6. *Bansod SD, Bawaskar MS, Gade AK, Rai MK. Development of shampoo, soap and ointment formulated by green synthesised silver nanoparticles functionalised with antimicrobial plants oils in veterinary dermatology: treatment and prevention strategies. IET Nanobiotechnol. 2015 Aug;9(4):165–71.*
7. *Shukla AK, Iravani S. Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier; 2018. 548 p.*
8. *Taylor & Francis Group. Pomegranates: Ancient Roots to Modern Medicine. CRC Press; 2020. 262 p.*
9. *Malkar VV, Mukherjee T, Kapoor S. Synthesis of silver nanoparticles in aqueous aminopolycarboxylic acid solutions via γ-irradiation and hydrogen reduction. Mater Sci Eng C Mater Biol Appl. 2014 Nov;44:87–91.*
10. *THE POMEGRANATE FRUIT GROWN IN SPAIN Antioxidant punicalagin in pomegranate juice and pomegranate extract, for the functional diet of the future. GRANATUM PLUS; 82 p.*
11. *Fernandes RA, Berretta AA, Torres EC, Buszinski AFM, Fernandes GL, Mendes-Gouvêa CC, et al. Antimicrobial Potential and Cytotoxicity of Silver Nanoparticles Phytosynthesized by Pomegranate Peel Extract. Antibiotics (Basel) [Internet]. 2018 Jun 26;7(3). Available from: http://dx.doi.org/10.3390/antibiotics7030051*
12. *Sparabombe S, Monterubbianesi R, Tosco V, Orilisi G, Hosein A, Ferrante L, et al. Efficacy of an All-Natural Polyherbal Mouthwash in Patients With Periodontitis: A Single-Blind Randomized Controlled Trial. Front Physiol. 2019 May 22;10:632.*

13. *Li Y, Yang F, Zheng W, Hu M, Wang J, Ma S, et al. Punica granatum (pomegranate) leaves extract induces apoptosis through mitochondrial intrinsic pathway and inhibits migration and invasion in non-small cell lung cancer in vitro. Biomed Pharmacother. 2016 May;80:227–35.*

14. *Umar D, Dilshad B, Farhan M, Ali A, Baroudi K. The effect of pomegranate mouthrinse on Streptococcus mutans count and salivary pH: An in vivo study. J Adv Pharm Technol Res. 2016 Jan;7(1):13–6.*

15. *Ghabanchi J, Moattari A, Darafshi R, Andisheh Tadbir A, Khorshidi H, Shakib M. Effects of three Commercial Mouth Rinses on the Cultured Fibroblasts: An in Vitro Study. J Dent. 2013 Jun;14(2):64–7.*

16. *Martins ML, Monteiro ASN, Guimarães JEC, Guimarães MB de CT, da Silva RF, Cabral LM, et al. Cytotoxic and antibacterial effect of a red propolis mouthwash, with or without fluoride, on the growth of a cariogenic biofilm. Arch Oral Biol. 2019 Nov;107:104512.*