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RESEARCH ARTICLE

Preparation of Mouthwash Using Musa Sapientum Mediated Silver Nanoparticles - Cytotoxic Effects

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ABSTRACT

Introduction: Musa sapientum belongs to the musaceae family and is most commonly known as banana. It is an edible fruit which is a large herbaceous and flowering plant seen in tropical and subtropical areas. It is used in the treatment of diarrhoea, dysentery and excess menstruation. It has properties such as antiulcerant, antibacterial, wound healing property, antiallergic. As it has properties like antimicrobial, antibacterial and bacteriostatic it can be used in the preparation of mouthwash.

Aim: To prepare mouthwash using Musa sapientum mediated silver nanoparticles and its cytotoxic effects.

Materials And Methods: 1g of Musa was measured and 100ml of distilled water was measured. Both were mixed together to make the aqueous extract. To that 10 nauplii were slowly added and the cytotoxic activity is analysed by the number of live nauplii counts.

Results: First day, Nauplii were grown in the medium. Nauplii hatch out after 24 hours. Second day, Mouthwash was added according to the concentration. Nauplii were collected and for each concentration 10 nauplii were added. After adding the nauplii, keep the cytotoxicity well aside undisturbed for one full day to analyze the inhibition of growth. Third day, nauplii were counted and cytotoxicity of mouthwash was evaluated.

Conclusion: Musa sapientem possess properties like anti bacterial, antimicrobial and bacteriostatic activity. It has good cytotoxicity and therefore it can be used in the preparation of mouthwash and can also be used commercially. Medicinal plants cure many diseases. Application of medicinal plants in the field of medicine should be improved.

KEYWORDS:
Musa sapientum; Mouthwash; Cytotoxicity; Nanoparticles; Silver nanoparticles; Herbal mouthwash

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INTRODUCTION

Musa sapientum belongs to the musaceae family and is most commonly known as banana. It is an edible fruit which is a large herbaceous and flowering plant seen in tropical and subtropical areas. Its native is Asian, Indo Malaysian and Australian. It is used in the treatment of diarrhoea, dysentery and excess menstruation (Ross, 2001). It has properties such as antiulcerant, antibacterial, wound healing property, antiallergic (Bährle-Rapp, 2007). Traditionally its fruit has been used for intestinal lesions, dysentery, diabetes, uremia, nephritis, gout, hypertension, and cardiac diseases. Leaves of banana are used in eczema as dressings for burns and blisters. Its flowers are used in dysentery (Barker and Steward, 1962). Stem juice of the banana plant is used for treatment of dysentery, diabetics and diarrhoea. Root part of the plant is

used as Anthelmintic, in treatment of blood disorders and in treatment of venereal diseases. The plant is also used in treatment in pain, inflammation and in snake bites (Sari et al., 2019). About 300 varieties of banana are cultivated in different countries. Bananas grow throughout the year, almost everywhere in the country (Ara, Basher and Hossain, 2012)(Shanmugam Rajeshkumar et al., 2021)(Ara, Basher and Hossain, 2012). It has a wide range of antimicrobial activity (Shree et al., 2020). Aqueous extract has been used as antimicrobial activity against Staphylococcus species and pseudomonas (Panda et al., 2018). Ethanolic and aqueous extract of banana reported to show good activity against Staphylococcus aureus. The aqueous extract of banana shows effective bacteriostatic activity (S and Satheesha, 2020)(Shanmugam Rajeshkumar et al., 2021) (S and Satheesha, 2020). It is necessary to use an antimicrobial agent which should have the ability to modify the oral environment by being effective only against the pathogens and without altering that normal flora of the oral cavity (Panda et al., 2020), (Karthik, Arivarasu and Rajeshkumar, 2020)(Shanmugam Rajeshkumar et al., 2021)(Karthik, Arivarasu and Rajeshkumar, 2020).

Mouthwash is an aqueous solution which has properties such as deodorant, antiseptic, refreshing properties and is mainly used for plaque control. Oral hygiene maintenance is done by controlling plaque and buildup of plaque, sticky film of bacteria and food that accumulates in the oral cavity (Barma et al., 2020). It can be prevented generally using toothpaste and toothbrush but the oral cavity can be effectively maintained using mouthwash. Mouthwash is a kind of solution that is used to reduce the microbes or microbial level in the oral cavity. It contains alcohol, sweeteners, synthetic agents, flavouring agents (Van der Weijden et al., 2015); (Desai et al., 2020).

Herbal products play an important role in controlling dental plaque and gingivitis. Natural products play a major role in drug development('Herbal Sources Used by The Public Against Infections', 2020). Oral candidiasis is a problem among immunocompromised patients, elderly people and people suffering from chronic illness (Banu, Nasreen Banu and Gayathri, 2016)(Shanmugam Rajeshkumar et al., 2021) (Banu, Nasreen Banu and Gayathri, 2016). WHO has recommended using natural products to overcome such problems and management of infections (Ahmad et al., 2018); (Balraj, 2020)(Shanmugam Rajeshkumar et al., 2021) (Balraj, 2020). Commercially produced mouthwash contains chemical compounds such as hydrogen peroxide, sodium lauryl sulphate, methyl salicylate and all these compounds are harmful to the buccal cavity. A herbal mouthwash or natural mouthwash is produced and has antibacterial and antifungal activities. Herbal mouthwash is in high demand as they possess all the properties and have less side-effects and also relieves the pain (Yadav, Mohite and Magdum, 2020) (Shanmugam Rajeshkumar et al., 2021)(Yadav, Mohite and Magdum, 2020); (Desai et al., 2020; Yadav, Mohite and Magdum, 2020)(Shanmugam Rajeshkumar et al., 2021)(Desai et al., 2020; Yadav, Mohite and Magdum, 2020).

Silver nanoparticles are best known for their antibacterial activity used in a number of applications ranging from disinfectants to water purification (Jaisankar and Arivarasu, 2020)(Devi, Subathra Devi and Gnanavel, 2014) (Gupta, Ariga and Deogade, 2018) (Saravanan et al., 2018) (Needhidasan, Samuel and Chidambaram, 2014). Nanoparticles are a viable alternative to antibiotics and seem to have a potential in solving the problem of multidrug resistance since ancient times (You et al., 2012). Silver has always been used as an antiseptic, antimicrobial against the bacteria due to its low cytotoxicity (Liao, Li and Tjong, 2019)(Shanmugam Rajeshkumar et al., 2021) (Liao, Li and Tjong, 2019). Silver nanoparticles interact with cell surfaces of various bacteria. Low concentration of silver nanoparticles reduces the toxicity and unpleasant taste. Nanoparticles are materials in the size that range from 1-100 nm; they are broadly applied in medicine, engineering and agriculture (Shanmugam Rajeshkumar et al., 2021). These are convenient and eco-friendly and used in place of physical and chemical methods (Zhang et al., 2014), (Barma, 2020)(Shanmugam Rajeshkumar et al., 2021)(Barma, 2020). Cytotoxicity is the nature of being harmful to cells. Brime SLA using the larvae of the crustaceans is a common method which is employed to analyse the cytotoxicity of bioactive compounds (Martínez-Gutierrez et al., 2012); (Shankar et al., 2020); (S. Rajeshkumar et al., 2021); (Kumar et al., 2021).Our team has extensive knowledge and research experience that has translated into high quality publications (Rajeshkumar et al., 2018; Nandhini, Rajeshkumar and Mythili, 2019; M. Gomathi et al., 2020; Rajasekaran et al., 2020; Vairavel, Devaraj and Shanmugam, 2020),(Santhoshkumar et al., 2019),(Raj R, D and S, 2020),(Saravanan et al., 2018),(Gheena and Ezhilarasan, 2019),(Ezhilarasan, Sokal and Najimi, 2018),(Ezhilarasan, 2018),((Dua et al., 2019; A. C. Gomathi et al., 2020; Vairavel, Devaraj and Shanmugam, 2020),(Ramesh et al., 2018; Duraisamy et al., 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Arumugam, George and Jayaseelan, 2021; Joseph and Prasanth, 2021) ,(Gnanavel, Roopan and Rajeshkumar, 2019),(Markov et al., 2021)

The present study aims at the preparation of mouthwash using Musa sapientum mediated silver nanoparticles and its cytotoxic effects.

MATERIALS AND METHODS

Extract preparation

1g of Musa sapientum was added in 100ml of distilled water. It was boiled for 10 to 15 minutes at 70°C. After boiling the plant extract was filtered using Whattmann's no:1 filter paper. In 250ml of conical flask and 60ml of 20 mM silver nanoparticles was prepared and 40ml of the plant extract was mixed. The flask was kept in a magnetic stirrer. The synthesised nanoparticles were preliminarily analysed by using UV visible spectrophotometer. The nanoparticle solution was centrifuged at 8000 rpm to prepare Nanoparticle pellets. The nanoparticle pellet was dried in the hot air oven at 80 deg celsius. The dried powder was sent for keratinisation and mouthwash was prepared.

Mouthwash preparation

0.3g sucrose, 0.001g of sodium benzoate, 0.01g of sodium lauryl sulphate dissolved in 10ml distilled water to that nanoparticle sample 600µL was added and flavouring agent peppermint 50µL was added.

Brine shrimp lethality assay-cytotoxicity

Saltwater preparation

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12ml of saline water was filled. To that 10 nauplii were slowly added to each well (5 µL, 10 µL, 20 µL, 40 µL, 80 µL, control). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours ELISA plates were observed and noted for number of life nauplii present and calculated by using a formula: No of dead napili / no of dead napili + no of live napili x 100

RESULTS

Extract and mouth wash preparation of Musa sapientum mediated silver nanoparticles

Fig.1: Preparation of mouthwash using Musa sapientum mediated silver nanoparticles

Cytotoxic Activity- Brine Shrimp Lethality Assay

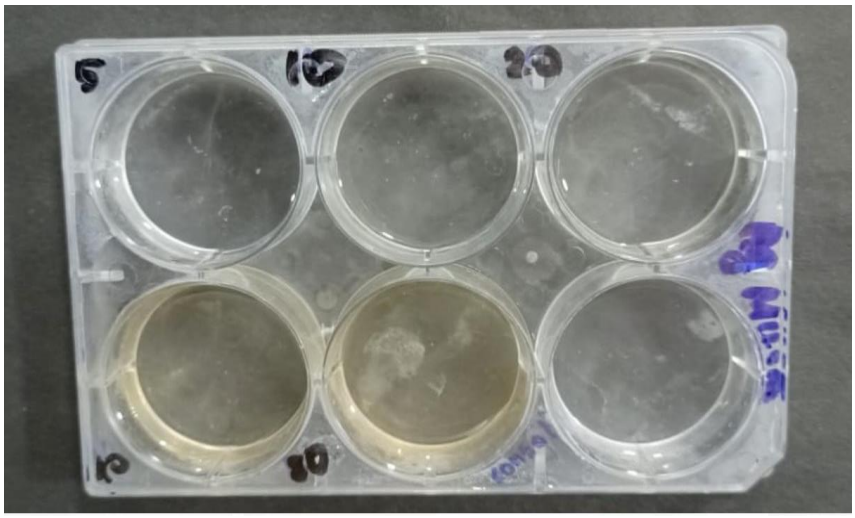

Fig.2: Wells showing the Cytotoxic activity of mouthwash using Musa sapientum mediated silver nanoparticles

First day, Nauplii were grown in the medium. Nauplii hatch out after 24 hours. Second day, Mouthwash was added according to the concentration. Nauplii were collected and for each concentration 10 nauplii were added. After adding the nauplii, keep the cytotoxicity well aside undisturbed for one full day to analyze the inhibition of growth. Third day, nauplii were counted and cytotoxicity of mouthwash was evaluated.

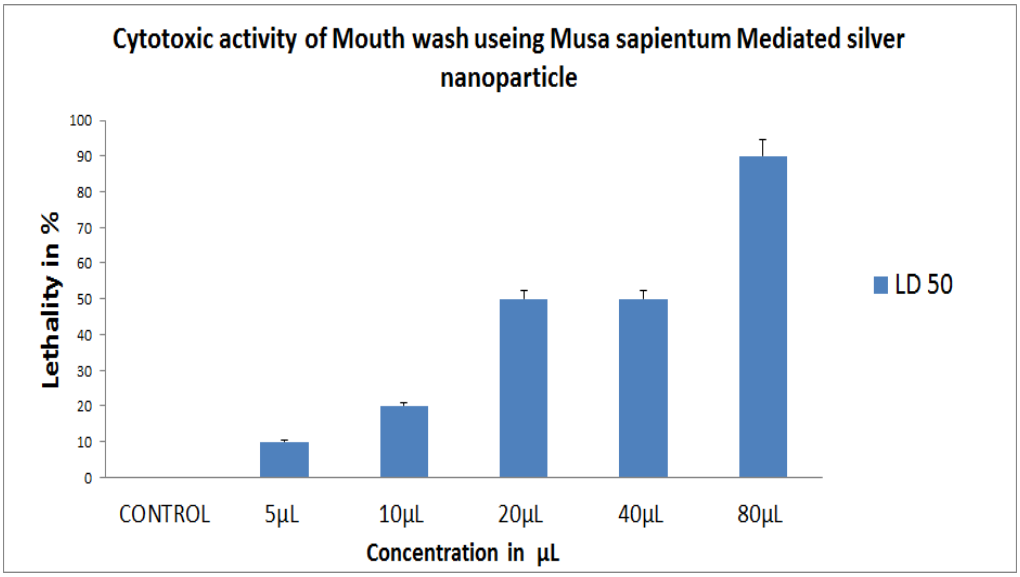

Fig.3 Cytotoxic activity of Mouthwash using Musa sapientum mediated silver nanoparticle, data implied as mean±SEM

At 5 µL, there is inhibition of growth of the nauplii. 10 nauplii was reduced to 9 at the end of 24 hours. At 10 µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 8 at the end of 24 hours. At 20 µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 5 at the end of 24 hours. At 40 µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 5 at the end of 24 hours. At 80 µL, nauplii growth was inhibited at this concentration. 10 nauplii was reduced to 4 at the end of 24 hours. Thus, the growth of inhibition was high at 80 µL concentration. Control was kept to avoid the confusion in the count of nauplii .

DISCUSSION

A previous study showed that the cytotoxicity of the enzymatic mouthwash was found to be lower than that of the chlorhexidine mouthwash. Expanded oxidative pressure was noticed for the mouthwash. Subsequent to presenting the fibroblasts to the mouthwashes, a G2/M stage block was noticed and cell death occurred transcendently by necrosis (Coelho et al., 2020). The present study showed the same that Musa sapientum based mouthwash has an cytotoxic effect against infective organisms which was done with larvae species, Nauplii. Cytotoxicity was determined by mitochondrial reductase action with essential gingival fibroblasts, L929 cells, and HSC-2 epithelial cells. Cells stayed fundamental after exposure to mouthwashes and were just utilized for cosmetic purposes. Moderate cytotoxic impacts were noticed for mouthwashes containing 0.05% chlorhexidine, ethanol, or pegylated hydrogenated castor oil and sodium dodecyl sulfate (Müller et al., 2017). Undiluted chlorhexidine mouthwash caused total cytotoxicity, similar to control groups. Further dilution of chlorhexidine mouthwash leads to increased rate of cell survival. Cytotoxicity increased with long exposure (Huang et al., 2000). Solutions which were tested completely inhibited the growth of microorganisms. It showed inhibitory activity against 24h-biofilm formation (Desai et al., 2020). Citronella oil has the lowest cytotoxic effect on dilution. It had a lower cytotoxic effect in comparison to commercial solutions (Cunha et al., 2020), (Rajaram et al., 2020). All evaluated mouthwashes like listerine, colgate, oral-B showed effective antibiofilm activity against oral pathogenic species like S aureus, S mutans, E coli, P aeruginosa, and C albicans, although the antiseptics were more effective on bacteria than on the yeast (Vikneshan et al., 2020). All the mouthwashes used were cytotoxic to gingival fibroblasts (Tabatabaei et al., 2020). This study was done as an in vitro representation of cytotoxic effects of Musa sapientum mediated mouthwash against a cellular organism(Pushpaanjali, Geetha and Lakshmi, 2020) (Aathira, Geetha and Lakshmi, 2020) (Baskar and Lakshmi, 2020) (Manya Suresh, 2020) (First Report on Marine Actinobacterial Diversity around Madras Atomic Power Station (MAPS), India, no date) (Physicochemical Profile of Acacia Catechu Bark Extract – An in Vitro Stud - International Journal of Pharmaceutical and Phytopharmacological Research, no date) (Lakshmi, 2021) (Awareness of Drug Abuse among Teenagers - International Journal of Pharmaceutical and Phytopharmacological Research, no date) (Mangal, Anitha and Lakshmi, 2018) (COX2 Inhibitory Activity of Abutilon Indicum - Pharmaceutical Research and Allied Sciences, no date) (Jibu, Geetha and Lakshmi, 2020) (Sindhu et al., 2020) (Nivethitha et al., 2020) (Mariona, Roy and Lakshmi, 2020). This study did not define the cytotoxic effect against pathogenic cells properly which is a limitation of this study and in future the cell line oriented in vitro study can be conducted to know the cytotoxicity of Musa sapientum against a cell which is cancerous or infective pathogen.

CONCLUSION

We conclude that with increase in the concentration, the number of dead nauplii increases. Therefore Musa mediated silver nanoparticles can be used effectively for mouthwash as it has potential cytotoxicity activity and are safe, eco-friendly and economical(Rajendran et al., 2019)(Ashok, Ajith and Sivanesan, 2017)(Malli et al., 2019)(Mohan and Jagannathan, 2014)(Menon et al., 2018)(Samuel, Acharya and Rao, 2020)(Praveen et al., 2001)(Neelakantan et al., 2011)('Oligonucleotide therapy: An emerging focus area for

drug delivery in chronic inflammatory respiratory diseases', 2019)(Kumar et al., 2006). Biological and environmental effects of silver nanoparticles must be studied further before it is commercially added into an available mouthwash.

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CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in the present study.

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