PREPARATION OF *BOERHAAVIA DIFFUSA MEDIATED* SELENIUM BASED MOUTHWASH-A COMPARATIVE MICROBIAL AND CYTOTOXIC EFFECT

Running title:A comparative study on microbial and cytotoxic effect of *boerhaavia diffusa* based mouthwash

Type of study

ABSTRACT

Introduction: The extract of *Boerhaavia diffusa* root, a plant used in Indian traditional medicine, has significant immunomodulatory Potential.Selenium,a nutrient element that has a massive function in biological systems ,is one of the the interesting compounds to integrate with antibacterial agents.Recently several studies have pointed out the ability of selenium nanoparticles to exhibit anticancer,antioxidant ,antibacterial and antibiofilm properties.

Aim: To analyze the antimicrobial and cytotoxic activity of *Boerhaavia diffusa* mediated selenium nanoparticles based mouthwash.

Materials and method: 1g of *Boerhavia diffusa* was added in 100 ml of distilled water. It was boiled; the plant extract was filtered using Whatman's no.1 filter paper. In 250 ml conical flask, 60 ml of 20 millimolar sodium selenite was prepared and 40 ml of the filtered plant extract was mixed. This flask was kept in a magnetic stirrer.The nanoparticle solution was centrifuged at 8000rpm to prepare nanoparticle pellets. The nanoparticle pellet was dried in a hot air oven at 80 degree celsius. The dried powder was sent for Characterization.A mouthwash is prepared.

The prepared mouthwash was tested for cytotoxic activity by brine shrimp lethality assay and antimicrobial activity evaluated the zone of inhibition of agar well diffusion method.

Results and Discussion: It was proved that cytotoxic activity of selenium bound mouthwash was less compared to the commercial mouthwash.The antibacterial activity of the selenium bound mouthwash against C albicans and S.mutans was significant when compared to the standard antimicrobial agent.

Conclusion: Based on the results ,this mouthwash has the required qualities to be commercially be used.Therefore further studies can be done to prove that this mouthwash can be used commercially.

Keywords: Mouthwash,antimicrobial activity,selenium based,*B.diffusa*,nanoparticles

INTRODUCTION

Plant extracts have been widely evaluated for possible immunomodulatory properties.The ethanolic extract of Boerhaavia diffusa root, a plant used in Indian traditional medicine, has significant immunomodulatory potential.[1]High incidence of bacterial infections and antibiotic resistance as a growing problem has urged the need for novel antibacterial agents.[2]*Boerhaavia diffusa* roots (Punarnava mool) are in use since the beginning of Ayurveda era for various therapeutic benefits. Root extract of this plant induces strong systemic resistance in susceptible host plant[3].*Boerhaavia diffusa* belonging to family Nyctaginaceae has a wide distribution, occurring on major parts of the globe.It is used as traditional medicine by indigenous people of many countries in the world for its protective role against inflammation, prostatic hyperplasia, diabetes, cancer, gastrointestinal problems, arthritis etc. The whole plant contains numerous bioactive compounds which are responsible for its pharmacological activities. Experiments are being done to evaluate full potential of the plant.[4] *Boerhaavia diffusa* contains a large number of phytoconstituents namely, flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, glycoproteins, punarnavine, boeravinone A–F, hypoxanthine 9-l-arabinofuranoside, ursolic acid, punarnava side, punarnavoside and liriodendrin.[5]

Cytotoxic agents are known as all the elements that are toxic to the cells, which include the factors that prevent their growth and sometimes cause death, and are also used to treat certain disorders. Chemical and biological substances or physical agents can cause cytotoxicity by

affecting the cells in varying degrees. Antimicrobial activity refers to the process of killing or inhibiting the disease causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal or antiviral.In this study the antibacterial and antifungal properties have been analysed.

In previous research to find the microbial activity of selenium particles,resistance of Escherichia coli and Staphylococcus aureus was compared in the presence of individual Nano and Bio counterparts as well as the nanohybrid system. Upon interaction of SeNPs with Lysozyme, the nanohybrid system efficiently enhanced the antibacterial activity compared to the protein. Therefore,it was concluded that SeNPs play an important role in inhibition of bacterial growth at very low concentrations of protein.[6].Selenium, a nutrient element that has a massive function in biological systems ,is one of the the interesting compounds to integrate with antibacterial agents.Recently several studies have pointed out the ability of selenium nanoparticles to exhibit anticancer,antioxidant ,antibacterial and antibiofilm properties.Oral mucositis (OM) is a complication of high-dose chemotherapy (HDC) followed by hematopoietic SCT (HSCT) with few effective treatments. Selenium has a cytoprotective role via the glutathione peroxidase (Glu.Px) enzyme and prevents chemotherapy-induced toxicities. It is involved in several key metabolic activities through selenoproteins, which are essential for protection against oxidative damage. In other words, selenium is a cofactor for glutathione peroxidase (Glu.Px), an endogenous enzyme system, which is able to scavenge free radicals. Some animal studies have shown that adequate supplementation of selenium could produce cytoprotective effects and anti-ulcer activity[7].Mouthwashes (MWs) have been particularly well accepted by individuals due to their ease of use. It is an effective method for delivery of antimicrobial agents thus preventing bacterial adhesion, colonization, and metabolism. However emergence of bacterial resistance to such agents has become a common phenomenon, which is represents a major problem.This has encouraged the development of alternative strategies to tackle drug-resistance problems.[8] Our team has extensive knowledge and research experience that has translate into high quality publications[9–20],[21–25]. [26] [27] [28][29–33]

In the present study the cytotoxic activity and microbial activity of the *B.diffusa* mediated selenium based mouthwash was analysed by comparing with the commercial mouthwash and

also the standard antimicrobial agents.This study could have also analyzed various other characters such as antioxidant, anti inflammatory activities etc.

MATERIALS AND METHOD

Extract preparation

1g of stem of *Boerhavia diffusa* was added in 100ml of distilled water. It was boiled for 10 - 15 minutes at 70 degree celsius. After boiling, the plant extract was filtered using Whatman's no.1 filter paper. In 250 ml conical flask, 60ml of 20millimolar sodium selenite was prepared and 40ml of the filtered plant extract was mixed. This flask was kept in a magnetic stirrer. The synthesised nanoparticles were preliminarily analysed by using UV visible spectrophotometer. The nanoparticle solution was centrifuged at 8000rpm to prepare nanoparticle pellets. The nanoparticle pellet was dried in a hot air oven at 80 degree celsius. The dried powder was sent for Characterization.A mouthwash is prepared.(Figure 1)

MOUTHWASH PREPARATION

0.3gsucrose,0.001g sodium benzoate,0.01g of sodium lauryl sulphate
Dissolved in 10ml distilled water. To that nanoparticle sample 600 microlitre was added.And flavouring agent peppermint oil was added- 50microlitres

BRINE SHRIMP LETHALITY ASSAY:Cytotoxicity test

Salt water preparation :

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.
6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL.80 µL,control). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula,

number of dead nauplii/number of dead nauplii+number of live nauplii×100

ANTIMICROBIAL TEST

Antimicrobial activity of respective nanoparticles against the strain staphylococcus aureus,E.faecalis, S.mutans and C.albicans MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and let them stabilize for solidification. The wells were cut using the well cutter and the test organisms were swabbed.The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition was measured.

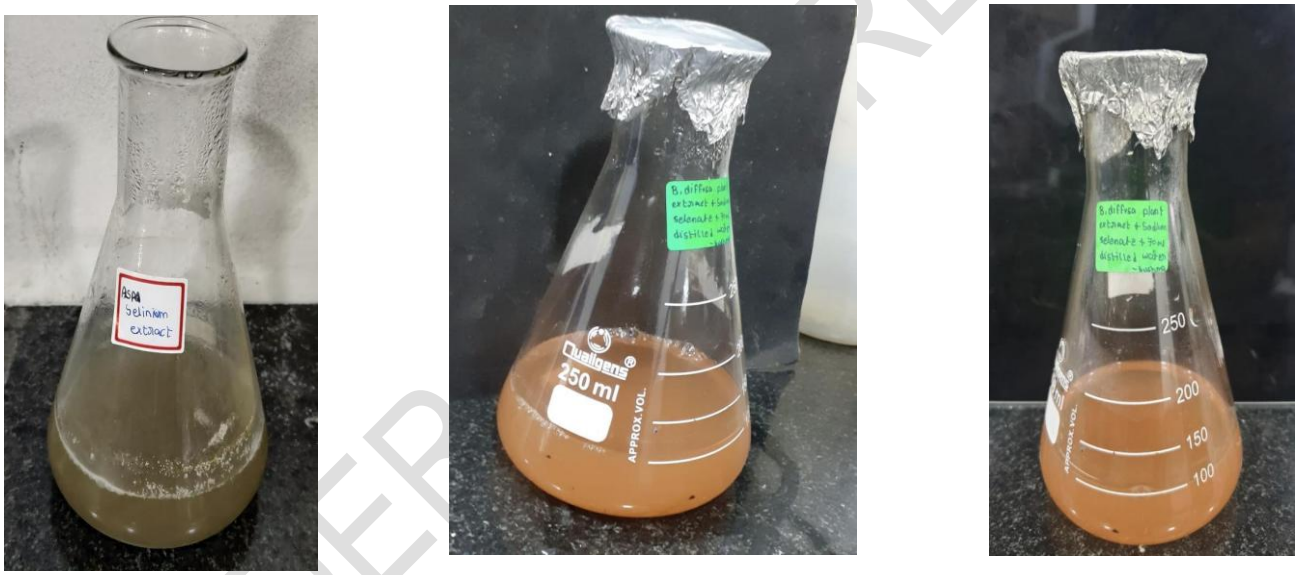


Figure 1:extract preparation and mouthwash preparation of B.diffusa mediated selenium nanoparticles based mouthwash.

RESULTS

The cytotoxic activity of the selenium based mouthwash was tabulated.The viability of the Nauplii was analysed for various selenium nanoparticles concentrations synthesised from mouthwash aided by *B diffusa*(figure 2).The nauplii in the well was noticed after 24 hours that

80% of Nauplii were alive in a minimum concentration of 5µL and 10µL. 90% of the Naupliiwere alive at 20µL and 40 µL concentration.At 80µL of concentrations 80% of the nauplii were alive.Whereas 100% of the nauplii were alive as shown by the control.Thus, according to other research, the increase in concentration enhanced cytotoxicity. In order to study the exact mechanism of action, more precise studies of cytotoxicity need to be performed. In the present analysis the cytotoxicity percentage was higher at 20µL and 40µLconcentrations.(Table 1)(graph 1)For comparing the selenium based mouthwash,commercial mouthwash was also analysed.It was noticed after 24 hours that 90% of the Nauplii were alive in the minimum concentration of 5µL and 70 % of the Nauplii were alive at 10µL ,20µL and 40µL.Only 50% of the nauplii were alive at 80µL concentration of the commercial mouthwash.Also at control 100% of the nauplii were alive.(table 2)(graph 2)Therefore as the concentration increases the number of nauplii decreases proving it to be highly cytotoxic whereas the selenium bound was less cytotoxic comparatively.The results were statistically analysed and was insignificant.p value was 0.226.(p value<o.o5)

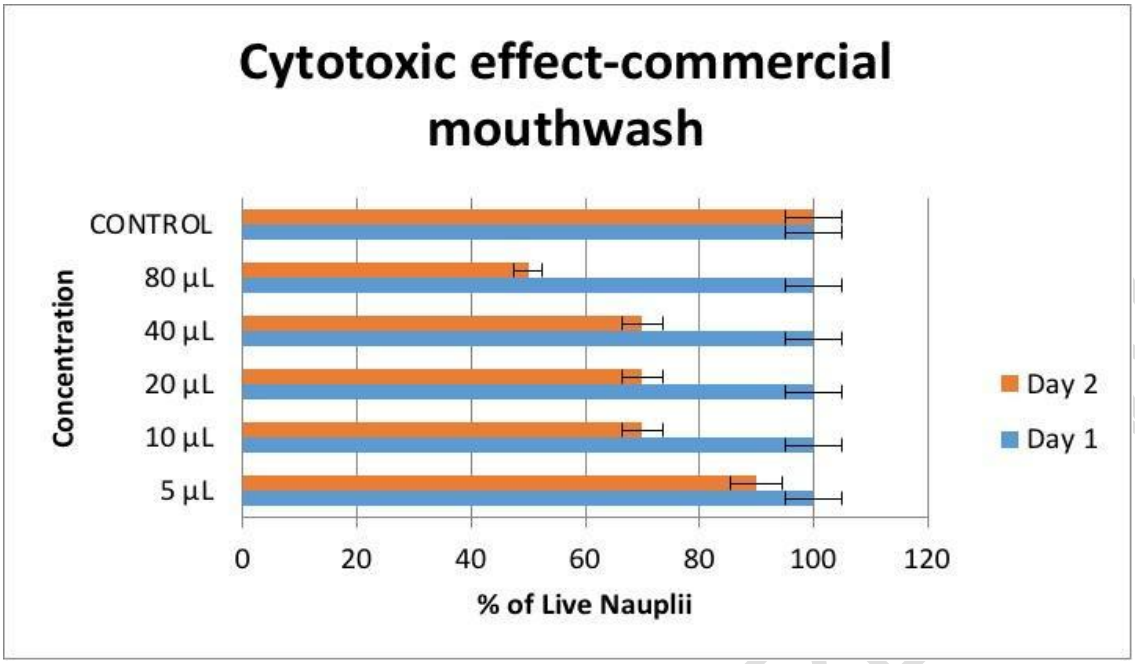
Cytotoxic activity

concentration	5µL	10µL	20µL	40µL	80µL	control
No of nauplii	8	8	9	9	8	10

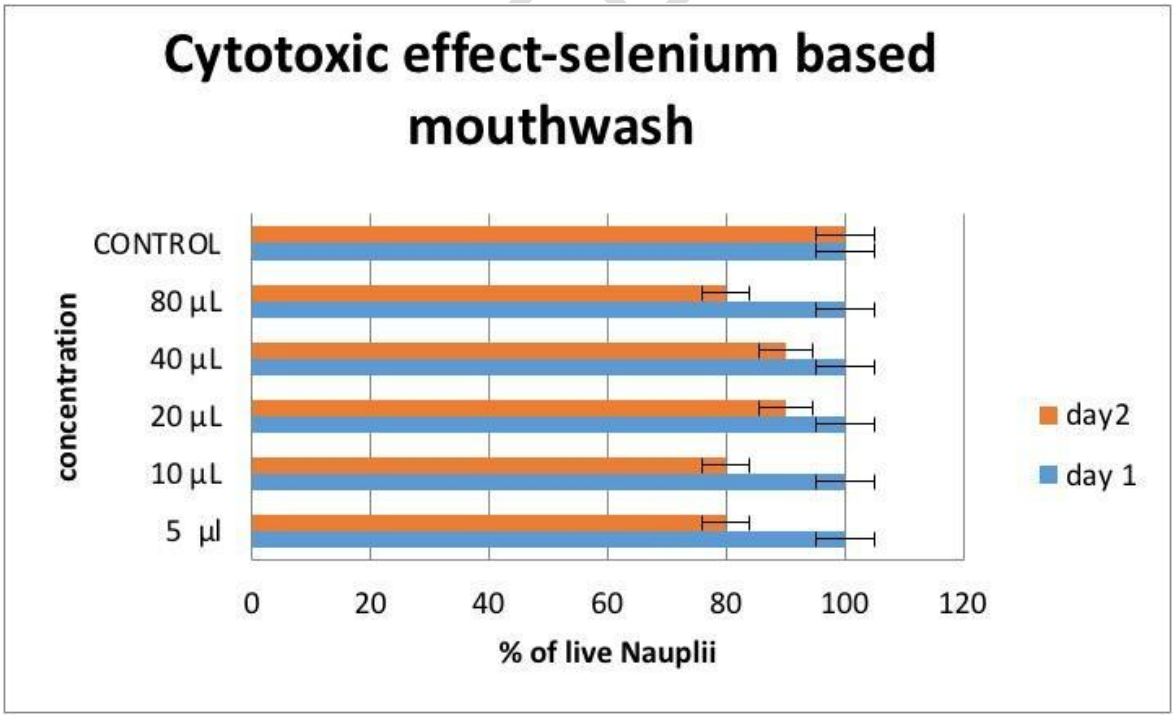
Table 1:Brine shrimp lethality assay for different concentrations of *B.diffusa* mediated selenium based mouthwash.

concentration	5µL	10µL	20µL	40µL	80µL	control
No of nauplii	9	7	7	7	5	10

Table 2:Brine shrimp lethality assay for different concentrations of commercial mouthwash observed after 24 hours



Graph 1:The bar graphs represents the cytotoxic activity of the commercial mouthwash.X axis depicts the number of live nauplii in percentage.Y axis represents the concentration of commercial mouthwash added.Blue represents day 1 and orange represents day 2.



Graph 2 :The bar graphs represent the cytotoxic activity of the selenium based mouthwash.

Figure 2:Brine shrimp lethality assay comparison ELISA plate well with different concentrations of selenium-based mouthwash and commercial mouthwash Observed for presence of live nauplii after 24 hours.

Antimicrobial activity

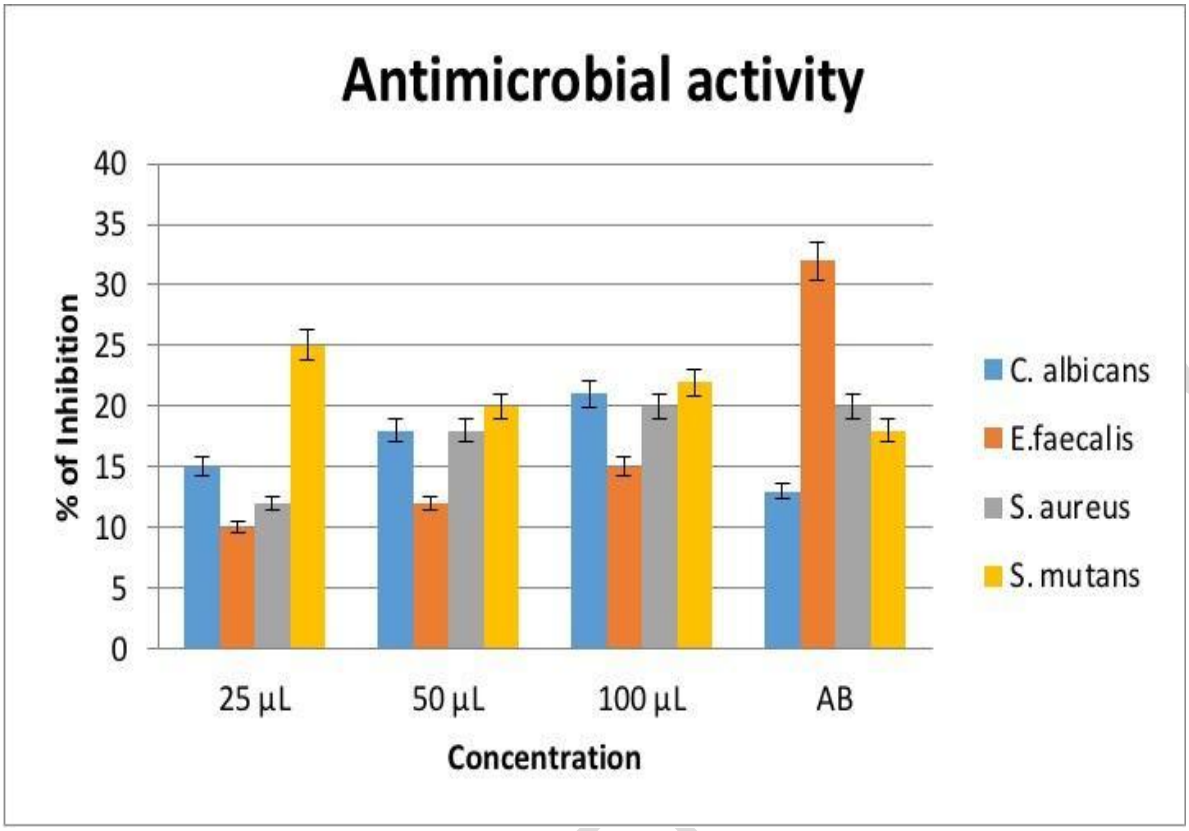
Table 3 demonstrates inhibition of bacterial growth at varying concentrations of biosynthesized selenium nanoparticles based mouthwash. The antimicrobial activity was evaluated based on their zone of inhibitions and the results were compared with the standard antibacterial agent. The antibacterial activity against S. aureus showed a zone of inhibition of 12mm at the concentration of 25µl.The antibacterial activity against S. aureus showed a zone of inhibition of 18mm at the concentration of 50µL.Antimicrobial activity against S. aureus showed a zone of inhibition of 20mm at the concentration of 100µL.The standard antibacterial agent had 20mm of zone of inhibition.Therefore comparatively the antimicrobial activity of selenium based mouthwash is moderate against S. aureus.The antimicrobial activity of selenium nanoparticles based mouthwash against S. mutans showed a zone of inhibition 25mm at concentrations of 25µL,20mm at 50µl and 22mm at concentration of 100µl.The standard antimicrobial agent has zone of inhibition of 18mm.Therefore this mouthwash when compared to the standard shows high antimicrobial activity The antimicrobial activity of selenium bases mouthwash against E. faecalis showed a zone of inhibition of 10mm at the concentration of 5µl.The antimicrobial activity of selenium nanoparticles based mouthwash against E. faecalis showed a zone of inhibition of 12mm at the concentration of 50µl.The antimicrobial activity against E. faecalis showed a zone of inhibition of 15mm at the concentration of 100µl.When compared to the

standard antimicrobial agent it is observed that this mouthwash has low level of antimicrobial activity against E. faecalis.The antimicrobial activity of selenium bases mouthwash against C. albicans showed a zone of inhibition of 15mm at the concentration of 5µl.The antimicrobial activity of selenium nanoparticles based mouthwash against C.albicans showed a zone of inhibition of 18mm at the concentration of 50µl.The antimicrobial activity against C.albicans showed a zone of inhibition of 21mm at the concentration of 100µl.(figure 3)When compared to the standard antimicrobial agent that is 13mm it is observed that this mouthwash has high level of antimicrobial activity against C.albicans.(table3)Therefore the antimicrobial activity of the B.diffusa mediated selenium nanoparticles based mouthwash was high against C.albicans and S.mutants.(graph 3).

B diffusa mediated selenium particles -microbial effect

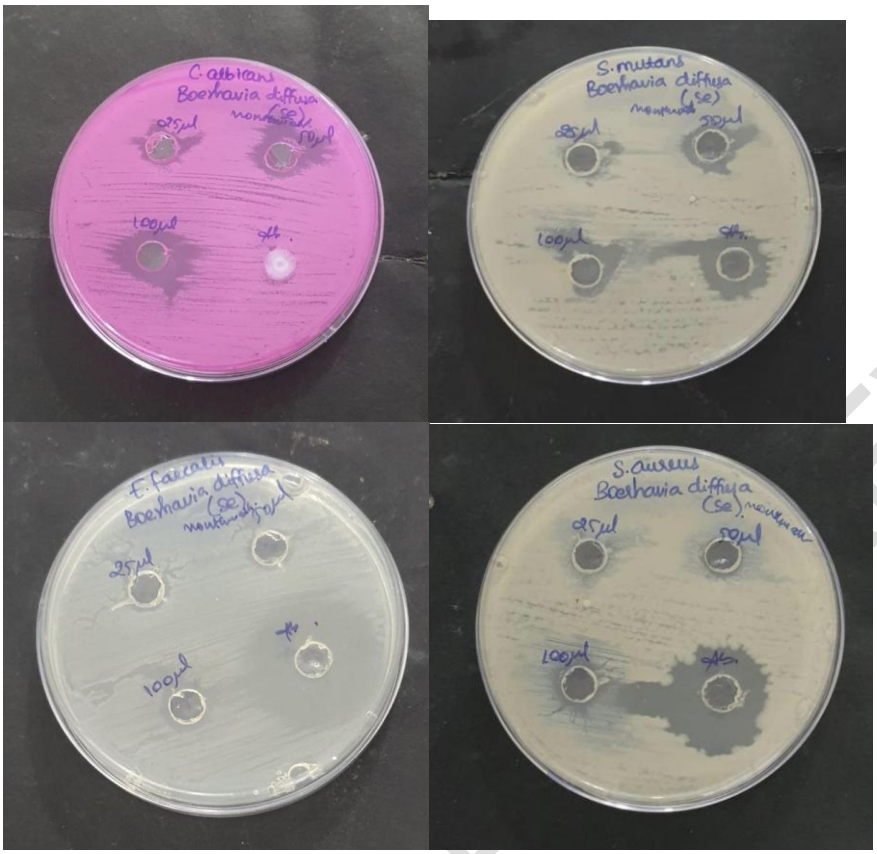
	25µL	50µL	100µL	AB
C albicans	15	18	21	13
E.faecalis	10	12	15	32
S aureus	12	18	20	20
S mutans	25	20	22	18

Table 3:Shows zone of inhibition of various oral pathogens at different concentrations.



Graph 3:The graph represents the antimicrobial activity of the selenium nanoparticles based mouthwash against C.albicans,E. Faecalis ,S.aureus and S.mutans in different concentrations and is compared with the standard antimicrobial agent.X axis represents the concentration of the selenium nanoparticles based mouthwash.Y axis represents the percentage of inhibition.

C.albicans S.mutans



E.faecalis S.aureus

Figure 3:Antimicrobial activity of B.diffusa mediated selenium nanoparticles based mouthwash.

DISCUSSION

In the analysis of toxicity, the brine shrimp lethality assay is an important test that provides us knowledge on the cytotoxic effect of a bioactive compound on cells [34][35][36][37].The viability of the Nauplii was analysed for various selenium nanoparticles concentrations synthesised from mouthwash aided by *B diffusa*(figure 2).The antimicrobial activity of selenium bound mouthwash that were synthesized with *Boerhaavia diffusa*,showed various zones of inhibition at different concentrations.[38][39][40][41][42] [43].

In previous articles, it has been observed that the cytotoxic activity of selenium particles was high which is not similar to the present study. Also The results of the previous study indicate that Selenium nanoparticles can be potentially used as antimicrobial and antioxidant agents similar to the present study[44]Many studies were done to analyze the cytotoxic activity and microbial activity of selenium particles but not many articles were published on selenium based mouthwash.[45].Brine Shrimp Lethality was done to check the cytotoxicity of nanoparticles and in previous articles it was found that there was increase of cytotoxic level with increased concentration of the administered nanoparticles which is unlike the present study[46].In a study done by vyas,the selenium nanoparticles were synthesised using garlic and he concluded that its antioxidant potential was high.[47] The present study only concentrates on cytotoxic activity and microbial activity which is a limitation of this study.Clove and cinnamon mediated selenium nanoparticles had high antimicrobial activity against C.albicans which shows similar results as that of present study.[48]Unlike the present study, the zone of inhibition was found to be high mainly for *S. aureus and E. faecalis*.[49]In this study mainly for S.mutans and C.albicans the zone of inhibition were found to high.Similar to this study ,The results of a article done previously showed that there was a lower toxicity rate of SeNPs.[50]

Only antimicrobial and cytotoxic activity of the mouthwash have been analyzed.Further more studies must be done to gain knowledge about the different characteristics of the *Boerhaavia diffusa* mediated selenium particles based mouthwash.Errors may occur while counting the live nauplii as well as in measuring the zone of inhibition of various microbes.

CONCLUSION

Within the limitations of the study we can conclude that *B.diffusa* mediated selenium nanoparticles based mouthwash had low cytotoxic potential and high antimicrobial activity.Therefore it is best to be used commercially.Further studies can be done to prove various other characteristics of this mouthwash to use this mouthwash commercially.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study.

AUTHOR CONTRIBUTION

Pravalika Arunkumar:Literature search,Data collection,analysis,manuscript writing

Geetha. R.V:Data verification,manuscript drafting

Rajeshkumar. S :Data verification,manuscript drafting

Ethical Approvals: we conducted our research after obtaining proper IEC approval.

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