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RESEARCH ARTICLE
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Biosynthesis of lemon seed conjugated silver nanoparticles lemon seeds and its anti-microbial activity against oral pathogens

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ABSTRACT

Introduction: Citrus fruits are native and abundantly grown in South Asia. They are good source of vitamin C and have for ever been in the medical books for their biological/biomedical importance and there is considerable evidence that citrus fruits may help reduce risk and return the growth of several serious diseases this research particularly aims to assess the anti-microbial activity of silver nanoparticles of lemon seeds on oral pathogens since this side is yet to be explored.

Materials and methods: The lemon seed was ground and prepared with silver nanoparticles using silver nitrate and centrifuged using micro pipette, the solution was tested on petri plates where oral pathogens were cultivated the final readings were taken and grab voice plotted using SPSS software.

Results and discussion: The results were overwhelming and almost as good as a standard antibiotic. This could potentially pave way to pharma industry since lemon seeds were never used before.

Conclusion: Silver nanoparticles with lemon seeds shows antimicrobial properties. Further in vitro and in vivo studies are needed.

Keywords: *Biosynthesis, pathogens, anti-microbial, nanoparticles*

INTRODUCTION

Lemon and other citrus fruits have for ever been in medical books. This is because of their Biomedical importance. Though, the pharma industries are producing abundant antimicrobial drugs. The bacteria have become resistant to them(1). This is because bacteria and microbes have the ability to develop resistance towards drugs used for the therapy(2). This creates a necessity for more antimicrobial drugs.

The lemon being one of the important citrus plant for its medical importance. It has been cultivated for its crude extracts which are significant against clinical bacterial stains(3). The flavonoids from the citrus how many, antifungal, antidiabetic, anti-cancer activities(4).

The crude extract from lemon is being extracted mainly from Pulp, peel, leaves etc. But the seed of the lemon was not yet explored. Having this in mind the present study aims at determining the antimicrobial activity of the silver nanoparticles using lemon seeds. There are several extraordinary studies on lemon and other citrus extracts being used for dental antimicrobial properties. Essential oils, tooth pastes, mouth rinses etc. have been formulated successfully(11)(12)(13).

Silver nanoparticles are set to enhance the antimicrobial and antioxidant properties. Incorporating silver nanoparticles enhance dental materials in respect to anti bacterial properties, because of their antiviral, antibacterial, and antifungal qualities, the Ag NPs, unlike other biomaterials used in dentistry, could be used in endodontics, restorative dentistry, periodontology, prosthetic dentistry, implantology, and oral cancers. Among the most remarkable nanoparticles employed in industrial and commercial applications are the Ag NPs. It has been heavily utilised in biological, technical, and antimicrobial goods. This narrative review gives a general overview of Ag NPs, covering their synthesis, antibacterial characteristics, usage in endodontics, restorative dentistry, and dental biomaterials, as well as any potential toxicity issues.(5)(9)(10)

MATERIALS AND METHODS

The lemon seed extract was prepared by drying and grinding the lemon seeds. 1g of grounded lemon seeds is mixed with 100ml of distilled water. For the silver nanoparticles silver nitrate was used. The lemon seed extract who is boiled for 10 minutes and 30ml of it was mixed with silver nitrate for silver nanoparticles. The final derive solution was kept at rest for three days for the nano particles to form. The colour of the solution was progressively getting darker, indicating the formation Of the nano particles. Then the conical flask was centrifuged as the final step.

Four oral bacteria were taken, Staphylococcus aureus, Enterococci faecalis, streptococcus mutans and Candida albicans. Streptococcus mutants being most prevailed oral bacteria.

Antibacterial Activity

Antibacterial activity ofrespective nanoparticles against the strain Staphylococcus aureus, Bacillus, and E.coli. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton agar were prepared and sterilize for 15 minutes at 121oC. Media poured into the sterilized plates and let stable for solidification. The wells were cut using 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentration (25µL, 50 µL ,100 µL)were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37 °C. After the incubation time the zone of inhibition were measured.

Antifungal activity

Candida albicans is used as test pathogen by agar well diffusion assay. Rose Bengal Agar is used to prepare the fungal medium. The prepared and sterilized medium was swabbed with test organism and nanoparticles with different concentration (25µL, 50 µL ,100 µL)were added to the wells and in the fourth well standard antibiotic flucanazole was loaded. The plates were incubated at 37°C for 48-72hours. After the incubation time the zone of inhibition were measured.

RESULTS

Zone of inhibition against S.mutans

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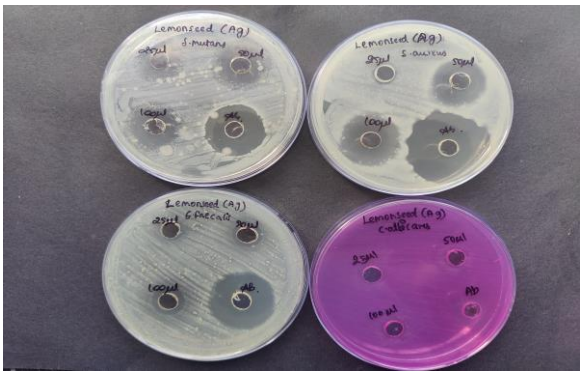
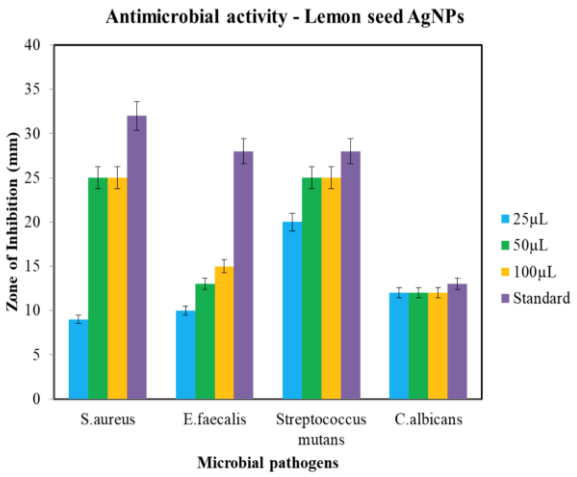
Zone of inhibition against E.faecalis



Zone of inhibition on the petri plate.

Zone of inhibition against C.albicans



Graph plotted using SPSS software to depict the zone of inhibition of each bacteria with different concentrations in comparison to standard antibiotic.

Zone of inhibition against S.aureus

DISCUSSION

We can observe that this zone of inhibition kept increasing in relation to concentration of lemon seed nano particles. This shows definite indication of presence of antimicrobial activity in lemon seeds. It was most effective on streptococcus mutants. It was almost as effective as standard antibiotic. The concentrations of the extraction taken were 25µL, 50µL and 100µL. 9mm, 25mm and 25mm of inhibition was shown on the culture plate for S.aureus. 10mm, 13mm and 15mm was shown for E.faecalis, compared to all the least antimicrobial action was shown on this. 20mm, 25mm and 25mm were exhibited for S.mutans. 12mm was constant on c.albicans and the standard drug showed similar as well.In one the studies in which citrus peel and pine apple was used to tackle the same they have concluded that significant antibacterial action against

cariogenic microorganisms including S. mutans and S. sanguinis was found in the peel of C. limon and pineapple.(6) They have achieved similar results as in one in the current study. There are similar studies to compare the current study to backup and validate the results obtained. A study conducted by Segata N, Haake SK, Mannon P, etal and another study conducted by Lemes RS, Alves CC etal based on essential oils derived from citrus peels also exhibit similar promising results.(7)(8). Our study shows a very strong action against S.aureus and S.mutans.

CONCLUSION

Herbal and natural medicines or from readily available resources, the growing interest of natural products in pharmaceutical and cosmetic industry needs to be met. From our study we can observe that silver nanoparticles from lemon seeds gave overwhelming results on oral pathogens specially on streptococcus mutans. Except for enterococci faecalis rest all were on par which standard antibiotic.

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