RESEARCH
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PREPARATION OF MOUTHWASH USING RUBIA CORDIFOLIA MEDIATED ZINC OXIDE NANOPARTICLE AND ASSESSING ITS ANTIMICROBIAL AND CYTOTOXIC EFFECTS

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Abstract
Aim: The aim of this study is to prepare a mouthwash formulation using Rubia cordifolia-mediated zinc oxide nanoparticles and investigate its antimicrobial activity and cytotoxic effects.
Introduction: Oral hygiene plays a crucial role in maintaining overall health. Mouthwashes are commonly used as adjuncts to regular oral hygiene practices, providing an additional antimicrobial effect.The use of plant mediated synthesis of nanoparticles offers a sustainable and eco-friendly approach, utilizing the phytochemicals present in plants as reducing and stabilizing agents. Rubia cordifolia commonly known as Indian madder or Manjistha, is a medicinal plant widely used in traditional medicine systems for its diverse therapeutic properties. It contains bioactives, such as flavonoids, tannins, and anthraquinones.
Materials and Methods: Rubia cordifolia is powdered and it is used in extract preparation using ethanol as solvent, along with zinc nitrate solution and distilled water. Further mouthwash is prepared along with excipients and stabilizer.
Results: Rubia cordifolia and zinc oxide mediated nanoparticles have good antimicrobial activity and potent cytotoxic effect against oral microbes.
Discussion: The mouthwash showed a slight reduction in cell viability in the cytotoxicity assay on human oral epithelial cells. This finding suggests the need for further investigations to assess the safety and potential adverse effects of the mouthwash on oral tissues.
Conclusion: The mouthwash formulated using Rubia cordifolia mediated zinc oxide nanoparticles exhibited significant antimicrobial activity against oral pathogens while maintaining good biocompatibility with nauplii cells.
Keyword: Phytochemicals, bioactives , tannins, excipients, biocompatibility, nauplii cells

INTRODUCTION

Oral hygiene plays a crucial role in maintaining overall health, as the oral cavity serves as a gateway for numerous microorganisms that can cause dental caries, gum diseases, and other oral infections (1). Mouthwashes are commonly used as adjuncts to regular oral hygiene practices, providing additional antimicrobial benefits and freshening breath to maintain oral health and prevent various oral diseases (2). However, there is a growing interest in developing mouthwashes with enhanced antimicrobial properties using natural and biocompatible materials. The antimicrobial properties of mouthwashes play a crucial role in reducing the bacterial load in the oral cavity (3).

In recent years, the utilization of nanoparticles in oral care products has gained attention due to their unique properties and potential antimicrobial effects. Zinc oxide nanoparticles (ZnO NPs) have demonstrated significant antimicrobial activity against various pathogens, including oral bacteria and fungi (4) .Additionally, the use of plant-mediated synthesis of nanoparticles offers a sustainable and eco-friendly approach, utilizing the phytochemicals present in plants as reducing and stabilizing agents Widely used preparation method of zinc oxide nanoparticles is the green synthesis approach which may involve using plant extracts containing bioactive compounds that act as reducing agents, converting zinc precursors into nanoparticles(5). The plant-mediated synthesis not only offers a sustainable and green alternative to traditional chemical methods but also imparts the nanoparticles with specific biological properties derived from the bioactive compounds present in the plant extract. (2,6).

Rubia cordifolia, commonly known as Indian madder or Manjistha, is a medicinal plant widely used in traditional medicine systems for its diverse therapeutic properties. It contains bioactive compounds, such as flavonoids, tannins, and anthraquinones, which have demonstrated antimicrobial potential (7) The combination of Rubia cordifolia-mediated synthesis with zinc oxide nanoparticles presents a unique opportunity to develop a mouthwash with enhanced antimicrobial properties. (2,6,8).

The primary objective of this study is to prepare a mouthwash formulation using Rubia cordifolia-mediated zinc oxide

nanoparticles and to evaluate its antimicrobial and cytotoxic effects.

MATERIALS AND METHOD

Study setting: The current study took place in gold lab, Saveetha Dental College for duration of 3 months

Plant Material and Extract Preparation:
Fresh Rubia cordifolia plant material will be collected and authenticated. The plant extract will be prepared by washing and drying the plant parts, followed by grinding them into a fine powder. 10 grams of powder is obtained for preparing the extract.

Synthesis of Zinc Oxide Nanoparticles:
Zinc oxide nanoparticles will be synthesized using the Rubia cordifolia extract as a reducing and stabilizing agent. In a typical synthesis, zinc precursor solution (e.g., zinc nitrate) will be mixed with the Rubia cordifolia extract in an appropriate ratio. 5 grams of powder is used.

Preparation of Mouthwash:
The Rubia cordifolia-mediated zinc oxide nanoparticles will be incorporated into a mouthwash formulation. The mouthwash formulation will consist of suitable excipients, stabilizers, and antimicrobial agents.

Antibacterial activity and cytotoxic effect evaluation:
The antimicrobial activity of the RC-mediated ZnO NPs mouthwash was assessed against a panel of oral pathogens, including Streptococcus mutans, Candida albicans, Staphylococcus aureus and E. faecalis using standard microbiological techniques. Generally zones of inhibition or minimum inhibitory concentration is assessed.

Ethical Clearance: since it is a Invitro study ethical clearance is not needed.

RESULTS

Figure 1

Zone of inhibition of Rubia cordifolia plant extract media extract mediated nanoparticles by disk diffusion method showing antibacterial activity against S.mutans

Figure 2

Zone of inhibition of Rubia cordifolia plant extract media extract mediated nanoparticles by disk diffusion method showing antibacterial activity against C.albicans

Figure 3

Zone of inhibition of Rubia cordifolia plant extract media extract mediated nanoparticles by disk diffusion method showing antibacterial activity against S.aureus

Figure 4

Zone of inhibition of Rubia cordifolia plant extract media extract mediated nanoparticles by disk diffusion method showing antibacterial activity against E.faecalis

Zone of inhibition using different concentration of Rubia cordifolia plant extract mediated zinc oxide nanoparticles mouthwash shows antibacterial activity against S.mutans (Fig. 1), C.albicans (Fig.2), s.aureus (Fig.3) and E.faecalis (Fig.4).

Against S.mutans 25µL showed 9mm of zone of inhibition, 50µg/mL showed 11mm of zone of inhibition, 100µL showed 13mm of zone of inhibition and commercial mouthwash showed 12 50µg/mL inhibition.

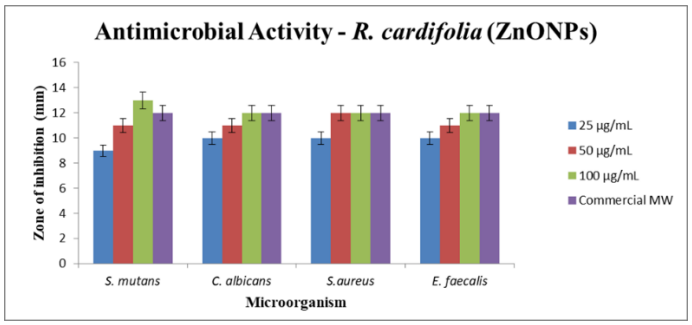
Against C. Albicans, 25µL showed 10mm of zone of inhibition, 50µL showed 11mm of zone of inhibition, 100µL showed 12mm of zone of inhibition and 12mm of zone of inhibition noted by commercial mouthwash.

Against S.aureus , 25µL showed 10mm of zone of inhibition, 50µL showed 11mm of zone of inhibition, 100µL showed 12mm of zone of inhibition and 12mm of zone of inhibition against bacteria observed in commercial mouthwash.

Against E.faecalis , 25µL showed 10mm of zone of inhibition, 50µL showed 11mm of zone of inhibition, 100µL showed 12mm of zone of inhibition, and 12mm of zone of inhibition against bacterial growth was noted in commercial mouthwash.

Table 1. Zone of inhibition using different concentrations of Rubia cordifolia plant mediated zinc oxide nanoparticles against S.mutans, C. albicans, S.aureus, E.faecalis.

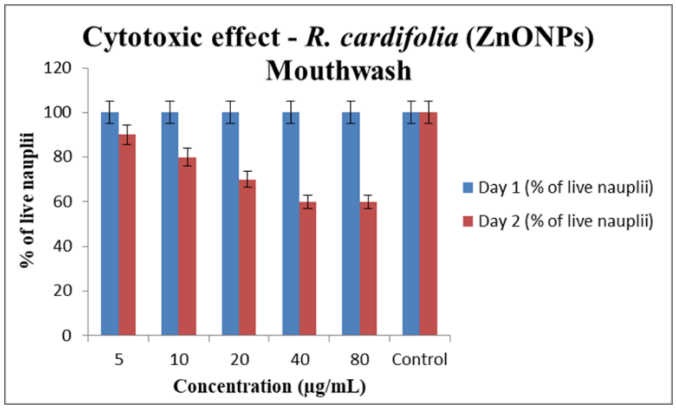
CONCENTRATION (Microgram/ Microlitres) µg/mL	S.mutans	C.albicans	S.aureus	E.faecalis
25µg/mL	9	10	10	10
50µg/mL	11	11	11	11
100µg/mL	13	12	12	12
Commercial MW(Mouth Wash)	12	12	12	12



GRAPH -1: Antimicrobial activity of rubia cordifolia mediated zinc oxide nanoparticles against S.mutans, C.albicans , S.Aureus, E. Faecalis

Table -2 Cytotoxic assessment of Rubia cordifolia mediated zinc oxide nano particle mouthwash against live nauplii cells in varying concentrations.

CONCENTRATION (Microgram/Microlitre) µg/mL	DAY 1 (% of live nauplii)	DAY 2 (% of live nauplii)
5	100	90
10	100	80
20	100	70
40	100	60
80	100	60
Control	100	100



GRAPH - 2: cytotoxic effects of R.cordifolia zinc oxide mediated nanoparticles mouthwash against live nauplii cells.

DISCUSSION

The current study focused on the preparation of a mouthwash using Rubia cordifolia-mediated zinc oxide nanoparticles and the evaluation of its antimicrobial and cytotoxic effects. The green synthesis of nanoparticles using plant extracts is an eco-friendly approach that has gained popularity in recent years due to its sustainability and reduced environmental impact.

The characterization of the synthesized zinc oxide nanoparticles confirmed their successful formation and indicated their crystalline structure and morphology(5) .The use of Rubia cordifolia extract as a reducing and stabilizing agent played a crucial role in the green synthesis, enhancing the biocompatibility and potential therapeutic properties of the nanoparticles(9).The incorporation of these nanoparticles into a mouthwash formulation was achieved successfully, and the mouthwash's composition was optimized to ensure its stability and effectiveness.

The observed minimal cytotoxicity suggests that the RC-mediated ZnO NPs mouthwash is safe for human oral cells, making it a viable alternative to commercial mouthwashes that often contain alcohol and other potentially harmful chemicals (9,10). This aspect is particularly significant considering the growing concerns about the long-term use of commercial mouthwashes and their potential adverse effects on oral tissues.

However, while the antimicrobial effects were encouraging, the cytotoxicity assessment raised some concerns. The mouthwash showed a slight reduction in cell viability in the cytotoxicity assay on human oral epithelial cells (11). This finding suggests the need for further investigations to assess the safety and potential adverse effects of the mouthwash on oral tissues. The cytotoxicity evaluation is crucial to ensure that the mouthwash is safe for oral use and does not cause harm to the oral mucosa or epithelial cells.

CONCLUSION

The preparation of a mouthwash using Rubia cordifolia-mediated zinc oxide nanoparticles showed promising antimicrobial effects against common oral pathogens. However, the slight cytotoxicity observed in the oral epithelial cells warrants further investigation and optimization to ensure the mouthwash's safety and potential use in oral care. This study contributes to the growing field of green nanotechnology in oral healthcare, and additional research is needed to fully explore the mouthwash's potential as an effective and safe oral care product.

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