GREEN SYNTHESIS OF ZINC OXIDE NPS USING BOERHAVIA DIFFUSA AND ITS ANTICARIOGENIC ACTIVITY

ABSTRACT:

INTRODUCTION:
Boerhavia diffusa (BD) is a plant of *rasayana* category as per ayurvedic claims. It is reported to possess anticariogenic activity, disease prevention, and life strengthening activities which hold enormous influence in disease burden and affordability/availability of healthcare in the world.
MATERIALS AND METHODS: 1g of Boerhavia diffusa is mixed 100 ml of distilled water and 2.5(0.514)g of (20 micromolar boerhavia diffusa) was dissolved in 60ml of distilled water to that 40 ml of filtered plant extract was added and was kept in an orbital shaker for approximately 72 hours. The formation of nanoparticles were confirmed both visually and by UV visible spectrophotometer. The nanoparticles were then centrifuged with the aid of a lark refrigerator centrifuge for 10 minutes at 8000 rpm.
RESULTS AND DISCUSSION: Anticariogenic activity of respective nanoparticles against the strain staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 min at 120Ibs. The media was poured into the sterilized playesa d was let to stabilize for solidification. The wells were cut using the well cutter and the test organism was swabbed.
CONCLUSION: Anticariogenic activity of respective nanoparticles against the strain staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 min at 120Ibs. The media poured into the sterilized plates was let to stabilize for solidification. The wells were cut using the well cutter and the test organism was swabbed.

KEYWORDS: B.diffusa, Green synthesis, Anticariogenic activity, Eco-friendly.

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INTRODUCTION:

Nanotechnology is the technological innovation of the 21st century. Research and development in this field is growing rapidly throughout the world (1). A major contribution of this field is the development of new materials in the nanometer scale. These are usually particulate materials with at least one dimension of less than 100 nanometers (nm), even the particles could be zero dimension in the case of quantum dots. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties(2). Nanoparticles exhibit completely new or improved properties with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles. Nanoparticles present a higher surface area to volume ratio with decrease in the size, distribution and morphology of the particles.The growing need for environmentally friendly nanoparticles, researchers are using green methods for the synthesis of various metal nanoparticles for pharmaceutical applications (3).

Boerhavia Diffusa (BD) is a well-known medicinal plant in traditional Indian medicine as well as other parts of the world, for example, the Southern American and African continent. Its various parts and especially roots have been used for gastrointestinal, hepatoprotective, and gynecological indications in above mentioned parts of the world and also throughout India. In ayurvedic, more than 35 formulations of different types contain it as a major ingredient (4,5). In Ayurveda, BD has been classified as “rasayana” herb which is said to possess properties like anti aging, reestablishing youth, strengthening life and brain power, and disease prevention, all of which imply that they increase the resistance of the body against any onslaught, in other words, providing hepatoprotection and immunomodulation (6,7).

Boerhavia Diffusa has been widely studied for its chemical constituents and therapeutic activities. The roots are the source of a novel class of isoflavonoids known as rotenoids, flavonoids, flavonoid glycosides, xanthones, purine nucleoside, lignans, ecdysteroids, and steroids (8,9). Various animal studies and trials have confirmed the presence of activities, for example, immunomodulation, hepatoprotection, antifibrinolytic, anticancer activity, antidiabetic activity, anti-inflammation, and diuresis (8). In this paper, traditional uses, chemical constituents, and reported pharmacological activities have been summarized to present the chemical and therapeutic potential of this plant (10).

The plants are easily available and safe to handle and the nanoparticles synthesized by plant extract are more stable, Green synthesis of ZNPs has been carried out by Boerhavia Diffusa. Boerhavia diffusa has also proven to be valuable managerial biosensing element synergy

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between green nanotechnology powerful biomedical agents (11)(12). Boerhavia diffusa used to treat accumulation of fluids are effective as they are called as Rasayana to treat Anemia and liver Diseases (9). Which acts as diuretic- anti inflammatory and hepatoprotective agents (13). They process anti- proliferative effects on cancer cells and prevent spreading (14)(15,16). Zno has a targeted drug delivery on - abri-cancer, anti-diabetic - antibacterial - antifungal and agricultural properties. Zinc oxide nanoparticles exhibit antimicrobial activity at micromolar concentration. Anti- cariogenic activities are the property of preventing Tooth decay or fighting cavities(17). Nanoparticles have multifunctional properties and have wide applications in various fields such as nutrition medicine and energy(18) (19) (20) (21). Various chemical methods have been projected for the synthesis of zinc (22,23). The anticariogenic activity of zinc oxide nanoparticles using boerhavia diffusa shows anti-growth and anti-adherence effects against cariogenic bacteria(24)(25,26).Our team has extensive knowledge and research experience that has translate into high quality publications(27–31),(32),(33),(20),(34),(35),(36),(29,37,38),(39–43),(44),(45).The objective of this study is to evaluate the anticariogenic activity of zinc oxide nanoparticles using boerhavia diffusa .

MATERIALS AND METHODS:

Preparation of plant extract : 1g of Boerhavia diffusa is mixed 100 ml of distilled water and 2.5(0.514)g of (20 micromolar boerhavia diffusa) was dissolved in 60ml of distilled water to that 40 ml of filtered plant extract was added and was kept in an orbital shaker for approximately 72 hours. The formation of nanoparticles were confirmed both visually and by UV visible spectrophotometer. The nanoparticles were then centrifuged with the aid of a lark refrigerator centrifuge for 10 minutes at 8000 rpm. The pellets were then separated from the supernatant and transferred into a single Eppendorf tube and stored for further studies.

EVALUATION OF ANTICARIOGENIC ACTIVITY:

Anticariogenic activity of Zinc oxide nanoparticle using Boerhavia diffusa against the strain of caries - causing bacteria organism like staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans were evaluated. MHA agar was utilized for this activity to determine the zone of inhibition using agar well diffusion method. Mueller-hinton agar was prepared and sterilized for 45 min at 120Ibs. The media was poured into the sterilized plates and was let to stabilize for solidification. The wells were cut using the well cutter and the test organism was swabbed. The nanoparticles (1mg/ml) with different quantities such as 25μl, 50μl and 100μl were loaded and the plates were incubated for 24 hours at 37 degree C. After the incubation time, the zone of inhibition was measured.

RESULTS :

Anticariogenic activity of respective nanoparticles against the strain staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 min at

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120Ibs(46). The media was poured into the sterilized playesa d was let to stabilize for solidification. The wells were cut using the well cutter and the test organism was swabbed(47). The nanoparticles (1 mg/ml) with different quantities such as 25µl, 50µl and 100µl were loaded and the plates were incubated for 24 hours at 37 degree C. After the incubation time, the zone of inhibition was measured (48).

FIGURE:1 Preparation of zinc oxide nanoparticle using boerhavia diffusa extract.

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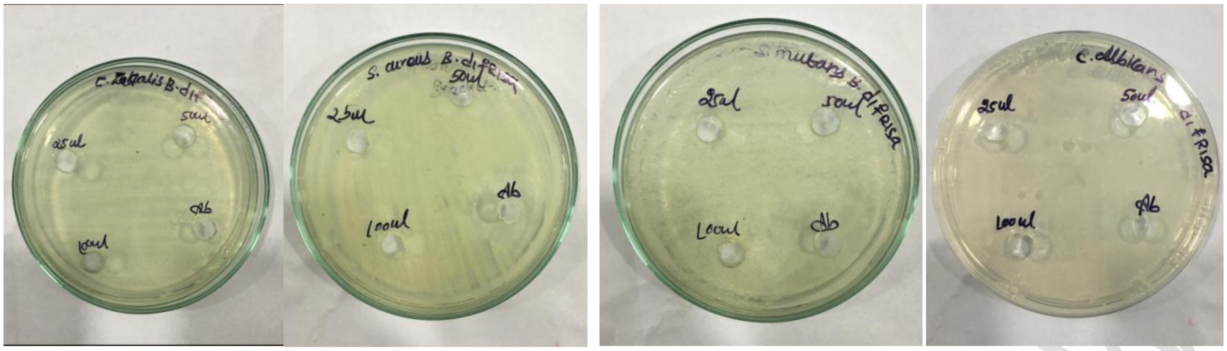
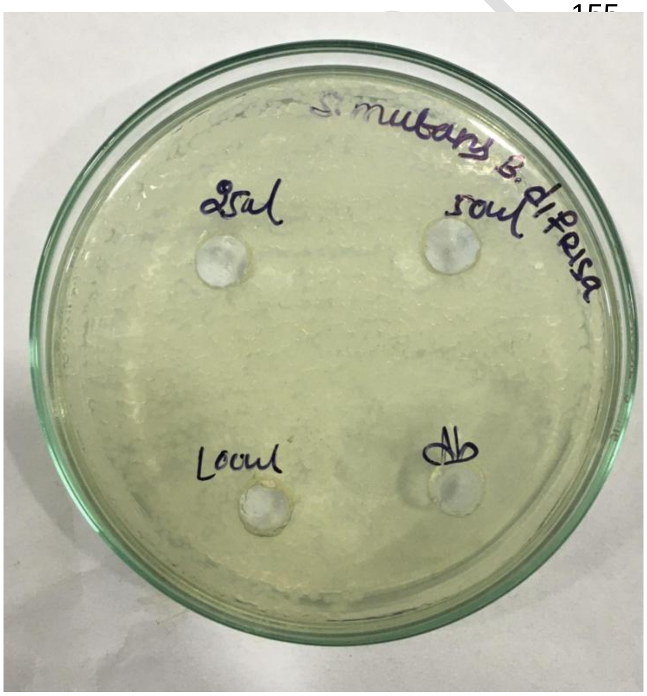


(A) (B) (C) (D)

FIGURE:2 Anticariogenic activity of zinc oxide nanoparticles using boerhavia diffusa (A)Enterococcus faecalis, (B) staphylococcus aureus, (C) staphylococcus mutans, (D) Candida albicans



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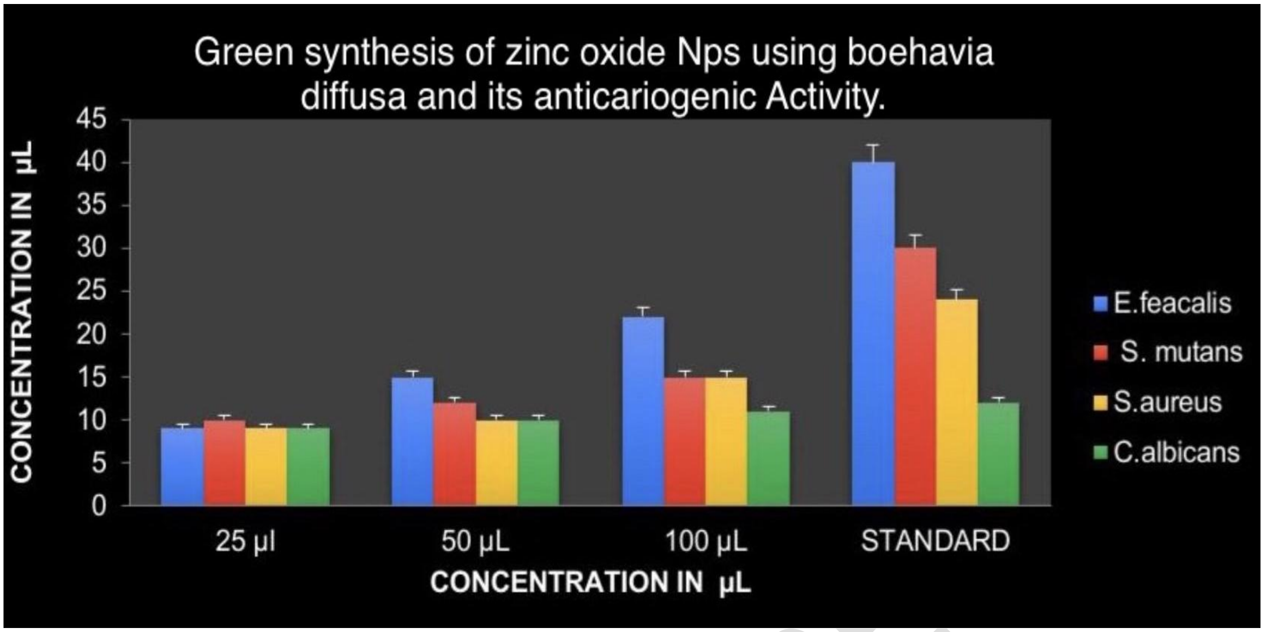

FIGURE:3 This figure represents the anticariogenic activity of zinc oxide nanoparticles using boerhavia diffusa. X axis refers to concentration in µl and, Y axis refers to the zone of inhibition of bacterias in mm, data were implied as mean ± SEM.

DISCUSSION:

The results obtained from the study were plotted in the form of graphs. The graph represents the zone of inhibition of *staphylococcus aureus*, *Candida albicans*, *Enterococcus faecalis* and *Streptococcus mutans* according to the concentration of the extract (48,49). At 25µl concentration, the zone of inhibition of staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans were 9 mm, 9 mm, 9 mm, 10 mm respectively. Whereas in the 50µl zone of inhibition were 10mm, 9 mm, 9 mm, 10mm. In 100µl the zone of inhibition were 11mm, 9mm, 9mm, 10mm, And at antibiotics the zone of inhibition of *staphylococcus aureus, Candida albicans, Enterococcus faecalis and Streptococcus mutans* were 24 mm, 12 mm, 42 mm, 40mm respectively (50).

The rapid biological synthesis of zinc nanoparticles using leaf extract of Boerhaavia diffusa provides an environmentally friendly, simple and efficient route for synthesis of nanoparticles (51). The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. The synthesized nano crystallites of ZnO are in the range of 30-35 nm (52).The synthesis of ZnO nanoparticles is still in its infancy and more research needs to be focused on the mechanism of nanoparticle formation which may lead to fine tuning of the process ultimately leading to the synthesis of nanoparticles with a strict control over the size and shape parameters (51,52). Infection is caused by a strain of Enterococcus faecalis bacteria that’s become resistant to the antibiotics commonly used to treat ordinary diseases (52,53)). MRSA infection may result in a number of clinical manifestations, including bacteraemia, endocarditis, sepsis, and death.

Given its resistance to therapy with multiple antibiotics, MRSA infection is often difficult to treat. A higher rate of biofilm formation is directly linked with the drug resistance pattern of MRSA (54). The ZnO nanoparticles coated surfaces could inhibit bacterial biofilm formation, thereby increasing the antibiotic exposure (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67) (68) (69)

The limitations of this study are that it can be done on various culture plates and observe the specific activity of each (70). It can also be done with many bacterial and fungal organisms excluding those that have been studied in this research (71)

The future scope for this study can lead to the development of commercial products of various nanoformulations, mouthwash, toothpaste, oral gels, etc that are safe, effective, and are economical (4)

CONCLUSION:

According to the present observation, we conclude that green synthesis of zinc oxide nanoparticles using boerhavia diffusa showed a good range of zones of inhibition and possessed excellent anticariogenic activity, especially against the *Enterococcus faecalis* . It is eco-friendly, effective, simple and powerful against multi-drug resistant bacteria(72)-(73). Zinc oxide nanoparticles can thus be used for traditional antibiotics as a non-toxic substitute.

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