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Analysis of Antimicrobial Activity of Aqua Alcoholic Extract of *Boerhaavia diffusa* Against Oral Pathogens -An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Boerhavia diffusa (Mukkirattai)* of the *Nyctaginaceae* family is a widely used folk medicinal plant that grows as a common weed with its leaves, seeds and roots are useful parts with pharmacological activities with a cure for twenty-three ailments. Phytochemical constituents of *B.diffusa* showing antimicrobial activity includes phenols, flavonoids, tannins, saponins, alkaloids, glycosides.
Aim: The present study aimed to evaluate the qualitative analysis of the antimicrobial activity of aqua alcoholic extracts of *Boerhaavia diffusa L.* (Family: *Nyctaginaceae*) leaves.
Materials and Methods: *Boerhavia diffusa* (*Mukkirattai*) was freshly procured as a powdered form from different solvent extracts that were tested against the Gram-positive bacteria and fungal strains by observing the zone of inhibition which showed the antimicrobial activity of *Boerhaavia*

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diffusa L. Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans and fungal strains *Candida albicans* were used in this test. The obtained data were analyzed statistically by Non parametric Spearman correlation analysis.

Results: Antimicrobial activity was observed in the aqueous-alcoholic extracts against Gram-positive bacilli, cocci and fungal strains. The aqua alcoholic extract of *B. diffusa* showed positive correlation with p value less than 0.05 with antimicrobial effect against fungal strains (e.g. *C. albicans* which had a zone of inhibition diameter of 26mm) and with Gram-positive bacteria (e.g. *Staphylococcus aureus* showed inhibition diameter of 20 mm), with Gram Positive cocci (e.g. *Streptococcus mutans* showed an inhibition diameter of 18 mm) when compared to *Enterococcus faecalis.*

Conclusion: The Aqua alcoholic extract of *Boerhavia diffusa* being biofriendly and inexpensive has a very strong Antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* while it showed minimal antimicrobial activity against *Streptococcus mutans* and *Enterococcus faecalis.*

Keywords: Boerhavia diffusa; antimicrobial; aqua alcoholic extract; green synthesis; zone of inhibition; nyctaginaceae.

1. INTRODUCTION

The *Boerhavia* genus distributed in the tropics of Africa, Asia and Australia has around forty species. *Boerhavia diffusa* of the *Nyctaginaceae* family is a widely used folk medicinal plant [1]. *B.diffusa* grows as a common weed with its leaves, seeds and roots are the useful parts with pharmacological activities with a cure for twenty-three ailments includes cardioprotective effect, treatment of prostatic hyperplasia, anti-inflammatory action, anxiolytic activity, protective effect on gastrointestinal problems, anticancer activity, antimicrobial activity, protection against harmful radiations, hepatoprotective activity, anti-arthritic activity and antidiabetic activity [2,3]. Other Indian names include varshabhu, Tambadivasu, Snathikari. It also helps in resolving abdominal pain, jaundice, diabetes, elephantiasis, ulcers, etc. [4]. Phenols, flavonoids, tannins, saponins, alkaloids, glycosides are the basic aqua-alcoholic phytochemical constituents of *B.diffusa* showing antimicrobial activity and in previous studies showed the presence of flavonoids, alkaloids, lignins, lipids, steroids, triterpenoids, proteins, carbohydrates and glycoproteins in *B.diffusa* extracts [5-7] Alcoholic and alkaline phytochemical flavonoids extract of *B.diffusa* is more compared to phenols and alkaloids [8].

The seeds of *Boerhavia diffusa* have anti-bacterial, anti-fungal and anti-pathogenic activity against *Enterococcus faecalis, Staphylococcus mutans, Staphylococcus Aureus, Candida albicans*. [9,10]. Antibiotics result in multidrug resistance in treating infectious disease also associated with anaphylactic reactions [11]. This forced the physicians to create new antimicrobial substances from medicinal plants with fewer hypersensitivity reactions. *Boerhavia diffusa* is a very good Ayurvedic medicine in India while it has Unani medicine properties which is also used Arabic countries for the treatment of various diseases like jaundice, inflammation, spleen enlargement ,heart failure, diabetes, stress and stress related factors, abdominal pain and dyspepsia [12-15]. It has also been reported that *Boerhaavia diffusa* is useful in the treatment of corneal ulcers, nephritic syndrome, elephantiasis, night blindness [16-18].

Plant-based antimicrobials are the source of medicines with multiple therapeutic potentials [19]. Both stem and leaf extract was tested against seven fungi species and six bacterial species, two types of yeast which showed antimicrobial activity in a dose-dependent manner (300-1800µg) and our research team has a exclusive knowledge and very vast experience which lead us into high quality publications [20-39]. In this present investigation, we have analysed the antimicrobial activity of aqua alcoholic extract of *Boerhaavia diffusa.*

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

Boerhavia diffusa (Mukkirattai) was freshly procured from Nature and Nurture health care pvt limited, New delhi as a powdered form which is the main advantage in our study. *Boerhavia diffusa* is a commonly available leaf in southern India and is well known for its health benefits. It is the best green medicine for diabetes. Aqueous-alcoholic extract was prepared in the nanotechnology lab of Saveetha Dental College

and Hospital. This original study protocol was approved and reviewed by the Research committee of Saveetha Dental College and Hospitals, Chennai, Tamilnadu, India.

For preparing an aqueous-alcoholic extract, 50 ml of ethanol is measured using a measuring cylinder. 5g of powdered *B. diffusa* was added to the 50 ml of ethanol and mixed well. Now the extract is transferred to the glass beaker and 50 ml of distilled water is added to the alcoholic extract. The beaker with aqueous-alcoholic extract of *Boerhavia diffusa* is covered with aluminum foil paper and the extract was mixed in an orbital shaker at 79.20 rpm. After 24 hours the extract was transferred to the measuring cylinder and the extract was boiled at 10% for 20 minutes and the extract was cooled at room temperature.

2.2 Antimicrobial Activity

2.2.1 Antibacterial Activity

Antibacterial activity of nanoparticles against the strain *Enterococcus faecalis, Staphylococcus aureus and Staphylococcus mutans*. Muller Hinton agar was mainly utilised for this, determining the zone of inhibition. Muller Hinton agar was sterilised and prepared for 45 minutes at 120lbs rate then the media poured into the sterilised plates was stabilised for solidification. Using the well cutter the wells were cut and the test organisms were swabbed. The plant extract at 25 µL,50 µL,100µL,150 µL concentrations were loaded and the plates were incubated at 37 ° C for 24 hours and the final zone of inhibition was measured after incubation. Manual labelling of the organisms should be given to avoid manual error.

2.2.2 Antifungal activity

Candida albicans are used as test pathogens by agar well diffusion assay. Sabouraud's dextrose Agar is used to prepare the medium. The prepared and sterilised medium was swabbed with test organisms and nanoparticles at various concentrations were added to the wells. The plates are incubated at 28° C for 48-72 hours. After the incubation time, the inhibition zone was finally measured and tabulated. The data obtained were tabulated and analysed by non-parametric spearman correlation analysis using SPSS version 23.

3. RESULTS AND DISCUSSION

The anti-microbial activity of aqua-alcoholic extract of *Boerhaavia diffusa* against *Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans and Candida albicans* summarised in Fig. 3. Results revealed that *Boerhaavia diffusa* showed antimicrobial activity of different magnitudes. The zone of inhibition at different concentrations (25 µL, 50 µL, 100µL, 150 µL) was done showing a reduction of the diameter of colonies around 26mm for *C.albicans*, 20mm for *S.aureus,* 18mm for *S.mutans* and 17mm for *S.faecalis.* Microorganisms are sensitive to different components of extracts of *Boerhaavia diffusa.* Spearman correlation analysis showed positive correlation (r=1) of decrease in zone of inhibition (mm) with increase in concentration and significant p value of less than 0.023. The sensitivity of bacterial and fungal species is observed in the following decreasing order *C.albicans>S.aureus >S.mutans>E.faecalis.*

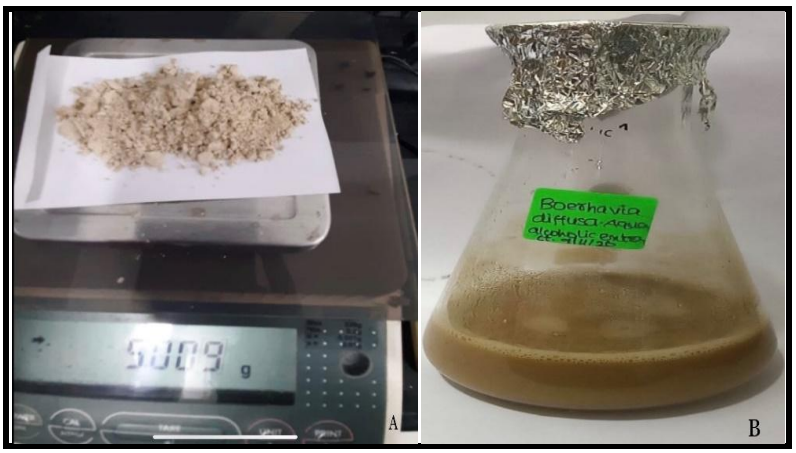

Fig. 1. Schematic representation of Preparation of *Boerhavia diffusa* extract. A-powdered extract of *Boerhavia diffusa.* B-Aqua Alcoholic extract of *Boerhavia diffusa*

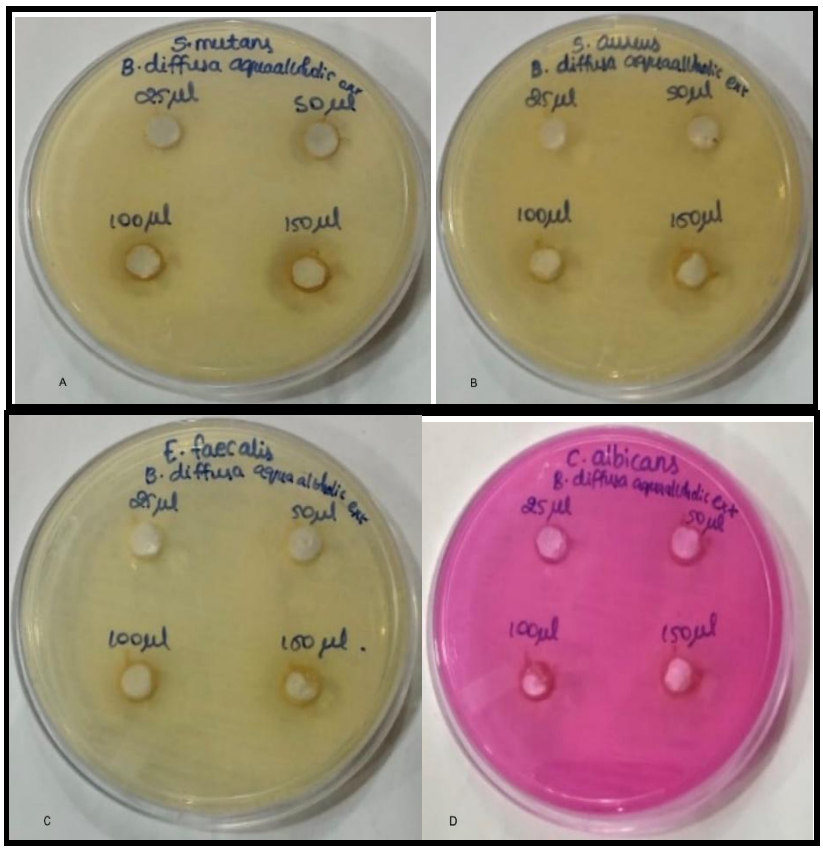

Fig. 2. Anti-microbial activity of *B. diffusa* extract on A - *Streptococcus mutans,* B - *Staphylococcus aureus,* C - *Enterococcus faecalis,* and D -*Candida albicans*

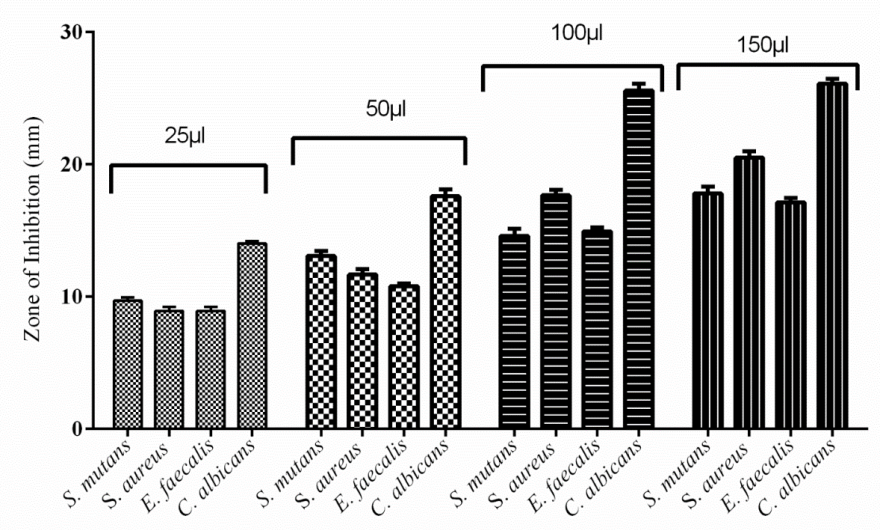

Fig. 3. Bar graph depicts the concentration of antimicrobial activity (x axis) of Aqua -alcoholic extract of *Boerhaavia diffusa* and the zone of inhibition (y axis). Positive correlation (r=1) with p value<0.05 is observed with increasing concentration of Aqua -alcoholic extract of *Boerhaavia diffusa*. Blue colour denotes staphylococcus faecalis, green represents staphylococcus aureus, grey represents streptococcus mutans, purple represents candida albicans. From this it is inferred that albicans showed maximum zone of inhibition with the least seen in faecalis

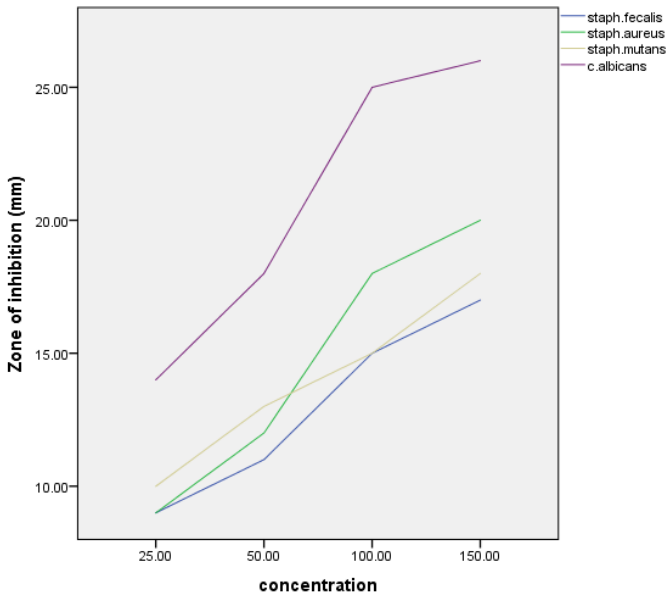

Fig. 4. Line Graph depicts the zone of inhibition of *Candida albicans* was higher with increase in concentration

Table 1. Depicts the zone of inhibition for *Enterococcus faecalis, Staphylococcus aureus, Streptococcus mutans and Candida albicans* at increasing concentrations

Concentration	25 µL	50 µL	100µL	150 µL
E.faecalis	9mm	11mm	15mm	17mm
S. aureus	9mm	12mm	18mm	20mm
S. mutans	10mm	13mm	15mm	18mm
C.albicans	14mm	18mm	25mm	26mm

In the present study, the zone of inhibition for *Enterococcus faecalis* at 25µL was 9mm, at 50µL it was 11mm and for 100µL it was 15mm and for 150µL it is 17mm. The zone of inhibition for *staphylococcus aureus* at 25µL is 9mm and at 50µL it is 12mm and at 100µL it is 18mm and at 150µL it is 20mm. The zone of inhibition for *streptococcus mutans* at 25µL is 10mm and at 50µL it is 13mm and for 100µL it is 15mm and at 150µL it is 18mm. The zone of inhibition for *Candida albicans* at 25µL is 14mm and at 50µL it is 18mm and at 100µL it is 25mm and at 150µL it is 26mm.

Bipin et al., study on antimicrobial activity of *Stevia rebaudiana and Azadirachta indica* against *S.mutans, E.foecalis, S.aureus and C.albicans* resulted with excellent antimicrobial activity against E.foecalis and S.mutans with a zone of inhibition of 35mm and 27mm [40]. Rotenoids are prototype compound named rotenone and isoflavonoids derivative a mitochondrial inhibitor causes ion inhibition electron transport chain in mitochondria at complex I, “toxophore” prenyl-derived ring of rotenoid structure and dimethoxy substitute to rotenone. Rotenoids isolated from *Boerhaavia diffusa* are noncytotoxic due to the lack of isoprenoid residue [41].

Staphylococcus and Streptococcus, Gram-positive bacteria invades skin, tissues, and bloodstreams. Coagulases, proteins activate prothrombin of the host, Surface of bacteria display proteins, agglutinins, and fibrin are the virulence factors of *S. aureus* infections leading to the destruction of immune cells, resulting in purulent exudates [42]. S. aureus has superantigen (SSL5&SSL10), prevents rolling and adherence of neutrophil along endothelium. S. aureus diminishes opsonization by targeting complement activation systems [43].

Candida albicans adhesion (adhesins) and invasion into host cells with hydrolase secretion, yeast hyphae transition, thigmotropism, phenotypic switching, altered pH biofilm formation (Hwp1 and Als3) Secreted aspartic

proteases (Saps) lead to endocytosis of C, Albicans. The utilization of lipases and amino acids results in hyphae formation. Hog1-, Mkc1-, Cek1-MAP kinase pathway responsible for maintaining the integrity of candida to the host surface [44]. *Candida albicans* adhere to the host surface with adhesin expression yeast-hyphae transition, growth by thigmotropism, Invasins mediate endocytosis of fungus into the host cell breaking down the barriers. Heat shock proteins, amino acids, lipases, ammonia excretion, and different trace compounds like zinc, carbon, manganese are responsible for hyphae formation.

Extract of *Boerhaavia diffusa* induced systemic resistance active component BDP-30 a glycoprotein, pI greater than 9.0 with amino acid sequence KLYDIPPLR is responsible for antimicrobial activity by inhibiting bacterial transduction between the host and the recipient cells also inhibits candida albicans biofilm formation by preventing dimorphism and switching of candidal hyphae formation [45]. The limitations of the study are constrained with four microorganisms at different concentrations. In future similar study in large scale productions for targeted drug delivery to treat and prevent a wide array of oral microbial infections.

4. CONCLUSION

The Aqua alcoholic extract of *Boerhavia diffusa* being biofriendly and inexpensive has a very strong antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* while it showed minimal antimicrobial activity against *Streptococcus mutans* and *Enterococcus faecalis.*

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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