Analysis of antimicrobial activity of aqua alcoholic extract of *Boerhaaviadiffusa* against oral pathogens -An Invitro study

ABSTRACT

INTRODUCTION

Boerhaviadiffusa(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 *Boerhaaviadiffusa L.* (Family: *Nyctaginaceae*) leaves.

MATERIALS AND METHODS

Boerhaviadiffusa (*Mukkirattai*) was freshly procured as a powdered form and the antimicrobial activity of different solvent extracts of *B. diffusa* were tested against the Gram-positive bacteria and fungal strains by observing the zone of inhibition. The Gram-positive bacteria used in the test were *Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans* and fungal strains *Candida albicans* were used. The obtained data were analysed 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*, the zone of inhibition diameter of 26mm) and against Gram-positive bacteria(e.g. *S. aureus*, the zone of inhibition diameter of 20 mm) and then Gram-positive cocci (e.g. *S.Mutans*, the zone of inhibition diameter of 18 mm) when compared to *E.faecalis.*

CONCLUSION

The Aqua Alcoholic extract of *Boerhaviadiffusa* has a very strong Antimicrobial activity against *Candida Albicans* and *Staphylococcus Aureus* while it showed minimal antimicrobial activity against *Enterococcus faecalis* and *Streptococcus mutans* and showed excellent potential as an antimicrobial agent and also as a biofriendly, inexpensive.

Keywords*Boerhaviadiffusa*, Antimicrobial, Aqua alcoholic extract, Green synthesis, Zone of inhibition, *Nyctaginaceae*

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Running title:Antimicrobial Activity of Aqua-alcoholic Extract of *BoerhaaviaDiffusa*

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INTRODUCTION

The *Boerhavia* genus distributed in the tropics of Asia, Africa, and Australia has around forty species. *Boerhaviadiffusa* 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, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins 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)]

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The seeds of *Boerhaviadiffusa*have anti-bacterial, anti-fungal and anti-pathogenic activity against *E.faecalis, S.aureus, S.mutans, C.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. *B. diffusa* is also used in Ayurvedic medicine in India and as an Unani medicine in Arab countries for the treatment of various factors like inflammation, jaundice, enlargement of spleen, congestive heart failure, diabetes, stress, dyspepsia, abdominal pain. ([12–15)] It has also been reported that *Boerhaaviadiffusa*is useful in the treatment of corneal ulcers, nephritic syndrome, elephantiasis, night blindness. [(16–18)]

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Several screening studies have been carried out in different parts of the world. 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 team has extensive knowledge and research experience that has translate into high quality publications [(20).(21–34) ,(35–39)] In this present investigation, we have analysed the antimicrobial activity of aqua alcoholic extract of *Boerhaaviadiffusa.*

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MATERIALS AND METHODS

Preparation of plant extract

Boerhaviadiffusa (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. *Boerhaviadiffusa* 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

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study protocol was reviewed and approved by the research ethical 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 *Boerhaviadiffusa* is covered with aluminum foil paper and then kept in an orbital shaker for general mixing of the extract 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.

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Antimicrobial activity

Antibacterial Activity

Antibacterial activity of respective nanoparticles against the strain *staphylococcus aureus, Enterococcus faecalis, and staphylococcus mutans*. 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 plant extract at 25µL, 50µL, 100µL, 150µLconcentrations were loaded and plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition was measured. Manual labelling of the organisms should be given to avoid manual error.

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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 sterilized medium was swabbed with test organisms and nanoparticles with different concentrations were added to the wells. The plates were incubated at 28° C for 48-72hours. After the incubation time, the zone of inhibition was measured and tabulated. The data obtained were tabulated and analysed by non parametric spearman correlation analysis using SPSS version 23.

RESULTS AND DISCUSSION

The anti-microbial activity of aqua-alcoholic extract of *Boerhaaviadiffusa* against *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and Candida albicans* summarized in Figure 3. The result revealed that *Boerhaaviadiffusa*showed antimicrobial activity of different magnitudes. The zone of inhibition at different concentrations (25µL, 50µL, 100µL, 150µL) 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*Boerhaaviadiffusa.* 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.*

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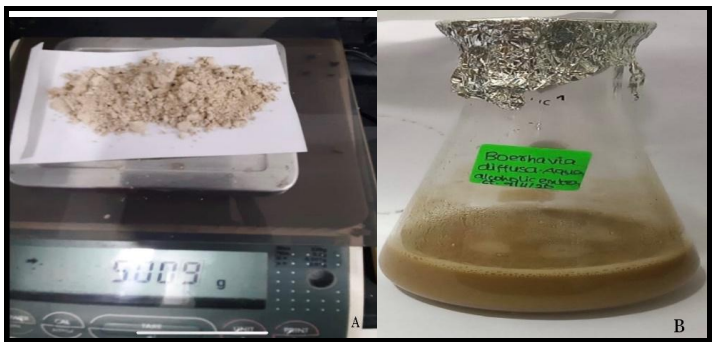

Figure 1 Schematic representation of Preparation of *Boerhaviadiffusa* extract. A-powdered extract of *Boerhaaviadiffusa*. B-Aqua Alcoholic extract of *Boerhaaviadiffusa*.

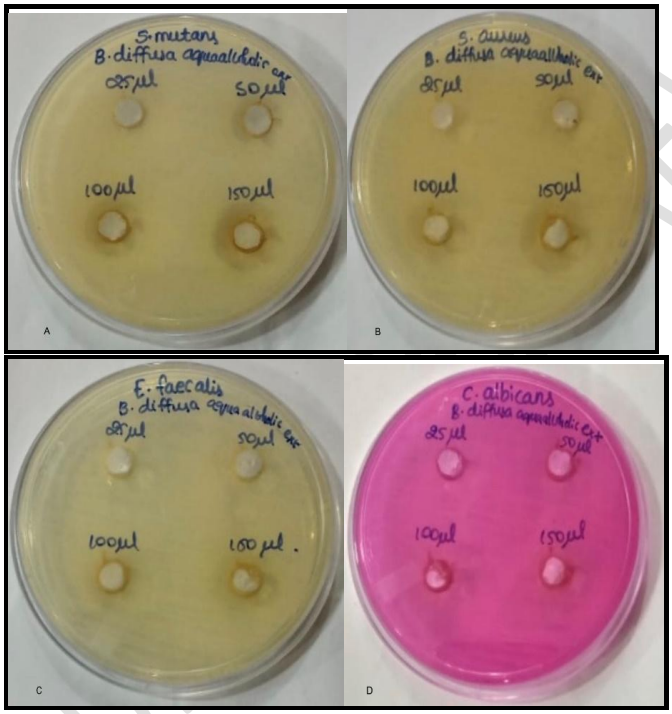

Figure 2 Anti-microbial activity of *B.diffusa* extract on A - *Streptococcus mutans,* B - *Staphylococcus aureus,* C - *Enterococcus faecalis,* andD -*C.albicans*

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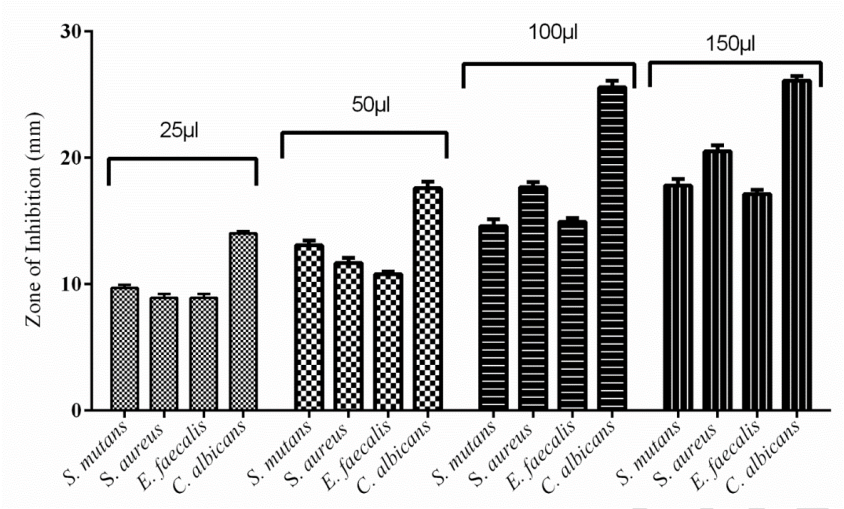

Figure 3: Bar graph depicts the concentration of antimicrobial activity (x axis) of Aqua - alcoholic extract of *Boerhaaviadiffusa*and the zone of inhibition (y axis). Positive correlation (r=1) with p value<0.05 is observed with increasing concentration of Aqua -alcoholic extract of *Boerhaaviadiffusa*.Blue 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.

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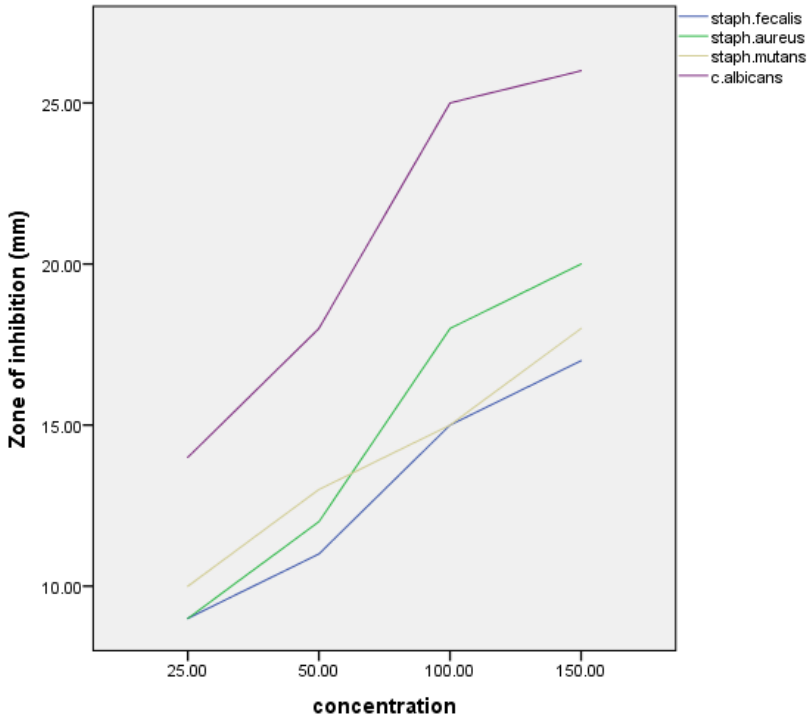

Figure 4 Line Graph depicts the zone of inhibition of *Candida Albicans* was higher with increase in concentration.

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TABLE-1 - Depicts the zone of inhibition for *Streptococcus mutans,Staphylococcus aureus,Enterococcus faecalis, 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.

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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 *Boerhaaviadiffusa*are noncytotoxic due to the lack of isoprenoid residue.[40]

Staphylococcus and Streptococcus, Gram-positive bacteria invades skin, tissues, and bloodstreams. Coagulases, proteins activate the hemostatic factor prothrombin of the host, Surface of bacteria display agglutinins, proteins and fibrin are the virulence factors of *S. aureus* infections leading to the destruction of immune cells, resulting in purulent exudate.[41] S. aureus has superantigen (SSL5&SSL10), prevents rolling and adherence of neutrophil along endothelium. S. aureus diminishes opsonization by targeting complement activation systems.[42]

Candida albicans adhesion(adhesins) and invasion into host cells with the secretion of hydrolases, 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. [43]*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 *Boerhaaviadiffusa* 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[44].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.

CONCLUSION

The Aqua Alcoholic extract of *Boerhaviadiffusa* has a very strong Antimicrobial activity against *Candida Albicans* and *Staphylococcus Aureus* while it showed minimal antimicrobial activity against *Enterococcus faecalis* and *Streptococcus mutans* and showed excellent potential as an antimicrobial agent and also as a biofriendly, inexpensive.

- **COMPETING INTERESTS DISCLAIMER:**
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- Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.
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