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Toxicological Evaluation Using Brine Shrimp (*Artemia salina* L.) Model and Antimicrobial Potential of *Barleria Prionitis* leaf Extracts on Microorganism of Hospital Origin

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ABSTRACT

The present research is first to investigate at the antibacterial and antifungal properties of *Barleria Prionitis* extract. Using the agar disc diffusion and microdilution methods. The most valuable method for monitoring the biological activity of various plant species is the brine shrimp lethality assay. This approach may be used to assess the toxicity of plant extracts in advance. The toxicity of a Brine Shrimp lethality test is usually measured after 24 hours of exposure to the examined sample. The Brine shrimp lethality assay is a great way to figure out how toxic plant extracts are. These *Barleria prionitis* extracts have potent antibacterial and may be used to treat diseases as an antibacterial.

Keywords: Barleria prionitis, Antimicrobial activity, Agar disc diffusion method, Acinetobacter lwoffii, Brine shrimp lethality assay.

INTRODUCTION

Ayurvedic and other traditional systems recognise the whole plant against a variety of disorders such as fever, cough, jaundice, and severe pain[1-2]. Recent pharmacognostical

screening reveals its efficacy as an active antioxidant, gastro-protective agent, and more as a significant source of secondary metabolites such as saponin, tannin, flavonoid, alkaloid, glycoside, and phenolic compounds. Nowadays, there is a growing interest in the development of plant-based drugs for the treatment of a variety of diseases. Furthermore, traditional drugs are being welcomed as a means of overcoming mild/serious illness. The emergence of resistant pathogenic microorganism strains has also raised concerns about the effectiveness of many medications, most notably antibiotics currently in use. Medicinal plants, identified as plants with one or more organs that contain substances that can be used for medicinal purposes or that serve as precursors in the production of drugs, are useful for disease treatment. Medicinal plants are worth investigating because they not only prevent people from experiencing pain but also allow them to survive it. Natural plants are commonly used as primary health remedies in Asia, Latin America, and Africa due to their pharmacological properties[3-5]. Microwave assisted synthesis is a novel method that contributes to property production by applying and extending the concepts of inexperienced chemistry. Many innovations that meet green chemistry goals already exist and can be used right away to reduce environmental pressures and improve economic efficiency[14-31]. Using batch or continuous-flow processing, current microwave reactors can convert small-scale microwave chemistry from mg to gm scale to multi-kilogram scale[32-48]. When processes are carried out in larger batch reactors, however, many of the advantages of small-scale microwave chemistry are lost. Incorporating green chemistry and related approaches into the education of current and future science students improves the efficacy of recruiting and retention initiatives in this critical area[49-62]. Both government and industry must invest in research beyond the current "pilot programme" levels in order to empower and allow the production and use of green chemistry technologies by a wide range of private-sector interests. Several new analytical methodologies have been defined that are fully compliant with inexperienced chemistry guidelines. They aid in the conduct of chemical processes as well as the study of their effects on the environment. The benefits of this enabling technology have also been utilised in multistep complete synthesis and medicinal chemistry/drug discovery, as well as related fields like polymer synthesis, nanotechnology, and biochemical processes. Green chemistry, also known as sustainable chemistry, has exploded in popularity since its inception a decade ago. Microwave synthesis will become an important part of and a common technology in most synthetic laboratories in the future, thanks to lower costs, and will continue to have a significant effect on both organic synthesis and drug discovery. It is the method of designing, developing, and implementing chemical products and processes in

order to minimise or eradicate the use and generation of substances that are harmful to human health and the environment. The cytotoxic effect of bioactive chemicals is now widely tested using the brine shrimp lethality assay[63-73]. This is a preliminary toxicity test of plant extracts. Following that, an animal model for establishment is suggested. Inhibition of crown gall tumours on potato tuber discs, frond proliferation inhibition in duckweed, and yellow fever larvae lethality test are some of the other top bench assays. This is a fast and accurate test for bioactive compounds, both natural and synthetic. It's also a low-cost and easy test since no aseptic techniques are needed[74-75].

MATERIALS AND METHODS

Plant Material

Barleria prionitis was obtained from Kasegaon, Sangli, Maharashtra, India.

Microorganisms

Inoculated on nutrient agar slats at 37°C were 4 gram-negative bacteria and one gram-positive bacteria and kept at -80°C.

Antibiotic Discs and Microdilution Assay

The disc diffusion method was used to test antimicrobial activity, with each microorganism having its own cell suspension. A 0.5 McFarland norm was used to equilibrate each cell suspension, and The Mueller–Hinton agar plates were distributed with 50 lL of the suspension of every micro-organism. In addition, 50 lL (1 g/5 mL of distilled water) diluted extract has been piped to sterile blank discs (6 mm in diameter) Dry on a biological laminar flow bench in an open, sterile Petri dish. Discs have been placed over inoculated plates and incubated at 37°C for 24 hours. Bacterial inhibition areas were measured in millimetres (mm) the disc diameter. Positive controls included cephalosporin discs. The plant extract's minimal inhibitory concentration (MIC) values against each investigated microbial strain were calculated using a microdilution assay in 96 multiwell microtiter plates, as per the Clinical and Laboratory Standards Institute's standard procedure (CLSI 2010). Mueller–Hinton Broth was used for all assays (MHB). The plant extract was dissolved to a final concentration of 10 mg/mL Dimethyl sulfoxide at 5 percent. Each line was tested for concentrations between 512.0, 256, 128, 64, 32, 16, 8,4, 2, 1, 0.5, 0.25, 0.12 and 0.06 lg/mL, with samples that had been serially diluted in the broth. The cultivation of each strain was given overnight, and the final concentration of the microorganism was adjusted to 106

CFU/mL in each well. The experiment was repeatedly duplicated by means of the results[76-82].

Statistical Analysis

Antibacterial activities were tested on the extract in triplicate. Using SPSS v. 11.5 and a fully random design.

Brine Shrimp Toxicity (BST) Assay

Preparation of seawater

38 gm of sea salt has been weighed, dissolved into one litre of water, and then filtered to obtain a clear solution.

Hatching of brine shrimp

The small tank was filled with seawater, and the eggs were pushed down and screened on one side of the tank. For two days, the shrimps could hatch and ripen like nauplii. A continuous supply of oxygen was provided during the hatching process. Egg shell-free nauplii have been collected by the lighted part of the tank because hatchy shrimps are attracted by light. The nauplii have been removed from the fish tank with a pipette and filtered to increase the visibility in clear, fresh seawater[83-84]. A 10 mg plant extract was analysed using an analytical balance[85].

Calculation

The percentage of nauplii lethality was determined. Calculate the percent death for each tube by counting the number of dead and live nauplii[86].

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$

RESULTS AND DISCUSSION

Antibacterial activity

Extract has the most influence on A. lwoffii and the least impact on P. aeruginosa. P. aeruginosa and A. lwoffii had been immune but vulnerable to extract cephalosporin. The MICs were 138, 40, 180, 95 g/mL respectively of A. lwoffii, A. aerogenes, K. pneumonia and S. aureus and of P. aeruginosa. Since P. aeruginosa and A. lwoffii were associated with the minimum and maximum MIC's, this result validated the results of the discussion. Medication could be jeopardised by antibiotic-resistant bacteria. A. lwoffii is a common organism,

relatively safe that can survive for long periods of time in a hospital setting.

Table-1: Mean diameters of inhibition zone

Microorganism	Inhibition zone diameter (mm)	
	Fruit extract	Cephalosporin
A. lwoffii	9 ± 0.06	0 ± 0.00
E. aerogenes	20 ± 0.19	6 ± 0.50
K. pneumoniae	16 ± 0.06	0 ± 0.00
S. aureus	18 ±0.50	5± 0.22
P. aeruginosa	28 ± 0.20	0 ± 0.00

Brine Shrimp Lethality Assay

With the aid of brine shrimp, extracts of Barleria prionitis were used in a cytotoxicity examination. Screening a wide variety of extracts for their various bioactivities is extremely beneficial to BSLA.

Table-2: BSLA of Barleria Prionitis

Sr. no	Test subs.	% death nauplii			
1	Methanolic extract	80	50	30	10
2	Ethyl acetate extract	70	20	30	10
3	Ethanolic extract	100	60	50	30
4	Aqueous extract	100	70	40	20

CONCLUSION

The antimicrobial activity of extract compared to broad-spectrum antibiotics like Cephalosporin on the microorganisms described above suggests the possibility of a more cost-effective and potentially harmless antimicrobial agent. The findings support the need for further research into the purification, detection, and quantification of active components, as well as the toxicity of active components, their side effects, and pharmacokinetic properties in order to use them in in-vivo studies. Although the bioactivity of plant extracts is very useful, the brine shrimp lethality test is somewhat ineffective when it comes to clarifying the mechanism of action. There was a pressing need to conduct in vivo animal model studies in order to collect precise data that could be extrapolated to humans. The BSLA approach

appeared to be a good one, particularly because in vivo research could still be categorised. Many other animal models have shown a clear correlation to Artemia Salina nauplii, and it is one of the options for biological toxicity research on herbal extracts. The preliminary knowledge of toxicity in the Brine shrimp lethality test provides a helpful platform for further toxicity testing.

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