

EVALUATION OF ANTIBACTERIAL EFFECT OF A NOVAL COMPOUND ISOLATED FROM *ELEUTHERINE BULBOSA* (MILLER) URBAN

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ABSTRACT

Eleutherine bulbosa belongs to family Iridaceae possess several medicinal properties. The underground bulbous part of the plant is used in the treatment of various ailments and considered medicinal under the local names, 'Neerotykihangu' or 'Vizhanarayani in Kerala. The present study deals with the antibacterial effect of a noval compound- bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal isolated from the bulbous part of the plant. The antibacterial activity of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal showed the lowest MIC value against *Staphylococcus aureus* (3.125µg/mL). The MBC value of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal against *Staphylococcus aureus* was 12.5µg/mL.

KEYWORDS: *Eleutherine bulbosa*, bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal, Iridaceae, antibacterial effect.

INTRODUCTION

Plants are the basis of life on earth. Besides the photosynthetic function, they act as sources of medicines also. Several plant derived drugs are now prevailing in the treatment of simple fever to deadly cancer. Considering the adverse effects of synthetic drugs, the whole world is looking forward for natural remedies, which are safe and effective. Herbal medicine is the oldest and still the most widely used systems of medicines in the world today.^[1] The Iridaceae is an ornamental plant family of about 85 genera and 1500 species, which has an almost worldwide distribution.^[2] Bulbous plants of Iridaceae family are commonly used as anticancer, antimutagenic, antiinfective, antimalarial, analgesic, immunostimulatory, respiratory and cardiovascular drugs.^[3,4,5,6,7] Investigations on the ethnomedicinal uses of

bulbous species can provide new insight for pharmaceutical developments. *Eleutherine bulbosa* is an important element of American Indian pharmacopoeia and is one of the few Neotropical Iridaceae with known medicinal uses.^[8] The underground bulb is considered medicinal under the local names, 'Neerotykhizhangu' or 'Vizhanarayani in Kerala. The crude ethyl acetate extracts of the plant showed potential antibacterial activity.^[9] A novel compound bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal was isolated from the crude ethyl acetate extract.^[10] The purpose of the present study is to evaluate the antibacterial activity of the above mentioned compound on four bacterial strains.

MATERIALS AND METHODS

Bioactivity studies

The compound bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal was subjected to antibacterial studies. The four human pathogenic bacteria used for the study were *Pseudomonas aeruginosa* (MTCC 741), *Proteus vulgaris* (MTCC 426), *Staphylococcus aureus* (MTCC 740) and *Salmonella typhi* (MTCC 733). The micro organisms were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The micro organisms were brought to laboratory conditions by sub culturing. The bacterial strains were grown in the nutrient broth at 37°C for duration 24h.

The Minimum Inhibitory Concentration (MIC) of the compound was determined using liquid dilution method.^[11] The bioassay was performed in 96 well sterile plates. The bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal was dissolved in 10% DMSO. The concentration of isolated compound used for the bioassays ranged from 400-0.025µg/mL. To each dilution, 50µL of the microbial culture, with 0.4 OD (Optical Density) at 620nm was added. Controls were prepared in the same manner, except that 50µL of broth was added in place of the microbial culture. The assay was done in triplicate and the test preparations were incubated at 37°C for 24h in a bacteriological incubator (KEMI BOD Incubator, Kerala, India). The MIC was determined by measuring absorbance of the test preparation at 620nm with a multiplate reader (Multiskan Ex, Thermo Electric Corporation, China) against the corresponding control. The lowest concentration which gave a zero absorbance reading was taken as the MIC of the test extract. The Minimum Bactericidal Concentration (MBC) of the compound was done according to the standard procedure.^[12,13] For this, 50µL aliquots of the test preparations which did not show any growth after incubation in MIC assays was added to

200 μ L of broth and incubated for specific periods. The lowest concentration which showed zero absorbance reading at 620nm was noted as the MBC.

RESULTS AND DISCUSSION

The MIC value of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) against the tested strains is shown in Table 1. The compound showed a lower MIC value against the tested strains. The MIC value is represented as follows *Staphylococcus aureus* (3.125 μ g/mL) > *Salmonella typhi* (6.25 μ g/mL) > *Proteus vulgaris* (50 μ g/mL) = *Pseudomonas aeruginosa* (50 μ g/mL). The MBC values against the tested strains are provided in Table 2. The lowest MBC value was shown against *Staphylococcus aureus* (12.5 μ g/mL) compared to other tested strains.

Table 1: MIC values of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal].

Micro organisms	bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal (μ g/mL)
<i>Pseudomonas aeruginosa</i>	50
<i>Proteus vulgaris</i>	50
<i>Staphylococcus aureus</i>	3.125
<i>Salmonella typhi</i>	6.25

Lower MIC value for the bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal was 3.125 μ g/mL.

Table 2: MBC values of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal.

Micro organisms	bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal (μ g/mL)
<i>Pseudomonas aeruginosa</i>	>100
<i>Proteus vulgaris</i>	>100
<i>Staphylococcus aureus</i>	12.5
<i>Salmonella typhi</i>	25

MBC values >100 μ g/mL were not significant.

Phytochemicals can be classified as antimicrobials based on their susceptibility tests which produce MIC values in the range of 100 to 1000 μ g/mL.^[14] Activity is considered to be significant if MIC values are below 100 μ g/mL and moderate when 100<MIC<625 μ g/mL.^[15,16]

The antibacterial activity of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal isolated from of *E. bulbosa* showed the lowest MIC value against *Staphylococcus aureus* (3.125 μ g/mL), which was found to be noteworthy. The MBC value of bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal against *Staphylococcus aureus* was 12.5 μ g/mL. The

MIC/MBC conferred by the bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal for *Staphylococcus aureus* was lower compared to crude extracts (MIC value= 9.7µg/mL, MBC value=39µg/mL).^[9] Evidence gathered from several studies showed that the partially purified isolated fractions exhibited significantly higher antibacterial activity compared to that exhibited by the crude extract against the tested strains. The column purified fractions of *Brillantaisia lamium* was found to be more active when compared to that the crude plant extract.^[17] The column purified crude extracts of *Cassia absus* leaves also showed comparatively higher antibacterial activity.^[18] The isolated fraction of *Dioscorea bulbifera* bulbils obtained using silica gel column chromatography with hexane: ethyl acetate as solvent system yielded antibacterial compounds.^[19] The isolated compound 8-hydroxyl naringenin from *Elephantopus scaber* showed potential antibacterial activity than crude acetone extract.^[20] The ethyl acetate fraction of crude methanolic extract of *Larrea tridentata* leaves showed significant antibacterial activity against *Staphylococcus aureus* (MIC value is 31.3µg/mL) than the crude methanolic extract (MIC value is 62.5 µg/mL).^[21] Noteworthy antimicrobial activities (MIC range between 2.00µg/mL and 16.00µg/mL) were observed for plumbagin isolated from *Aristea ecklonii*.^[22]

CONCLUSION

The bioactivity studies of bis (2-6, 7-diamino-5, 8-dioxo naphthalene-1yl) propanal showed that it possess antistaphylococcal activity. The compound is of plant origin and can be used in very low concentrations, which will open new horizons in the field of phytomedicine. However further preclinical and clinical trials are required for this compound to be finally used as a therapeutic medicine.

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