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Original Article

Bactericidal Activity of Physagulin F isolated from *Physagulin angulata* Fruit Extract

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ABSTRACT

Background: Physalis angulata, (Solanaceae), a branched annual shrub is commonly known as camapu or balaozinho in Brazil, belongs to Solanaceae. It is majorly distributed in tropical and subtropical regions of the world. *Physalis agulata* possess wide range of medicinal applications. *Aim:* The current study was framed out to evaluate the bactericidal activity of Physagulin F isolated from fruit extracts of *Physalis angulata. Methods:* The assay was conducted to assess the bactericidal activities of plant compounds through microtiter plate method. Gram positive and gram negative strains were used. *Results:* Bactericidal efficacy of Physagulin F was found high against Methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis*. On the other hand, among Gram negative strains *Escherichia coli* was noticed high susceptibility. The bactericidal activity of physagulin F was found high for first 3-12 hrs and followed by plateau in activity during the next 12-24 hrs. We have noticed that for most of the screened bacterial strains, the susceptibility was initiated after 6 hrs. whereas, some of the bacterial strains viz., *MRSA, E. coli, B. Subtilis* showed susceptibility within 3 hrs of incubation *Conclusion:* The results from the current study are very encouraging and indicate this compound should be studied more extensively to explore its potential antioxidant and antiinflammatory properties as well

Keywords: Physalis angulate, Physagulin, Bacteriocidal, antioxidant.

1. INTRODUCTION

Physalis angulata, (Solanaceae), a branched annual shrub is commonly known as camapu or balaozinho in Brazil, belongs to Solanaceae.[1]. It is majorly distributed in tropical and subtropical regions of the world. Physalis *agulata* possess wide range of medicinal applications. The extracts or infusion of this plant is used in the treatment of asthma, hepatitis, malaria, dermatitis, rheumatism, anticancerous, antimycobacterial, anti-tumor, hypotensive, immune stimulant, anti-coagulant etc. [1,2-6] The fruit and aerial parts

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are extensively used in the treatment of constipation, sores, boils, cuts, intestinal and digestive problems.[7-8] *In vivo* anti-tumour activity of this plant extract was demonstrated in mice.[3, 9] Physalis angulata L is one of such medicinal plant which is frequently used in the treatment of gonorrhea.[10] Root aqueous extract of this plant is reported possess antinociceptive activity.[11]

Methanol extracts of *Physalis angulata* reported significant antiperidontic property, anti-inflammatory and immunomodulatory activities. [11-12] This plant extracts also used as anti-mutagenic, anti-leucemis and anti-spasmodic, agents.[13] In context to wide range of medicinal applications of the crude extracts of this plant, it is urgent to determine the biological activity of compounds isolated from *Physalis angulata*. Hence, the current study was framed out to evaluate the bactericidal activity of Physagulin F isolated from fruit extracts of Physalis *angulata*.

2 MATERIALS AND METHODS

2.1 Bacterial Strains

Gram positive Strains Methcillin- resistant *Staphylococcus aureus* (MRSA, NCTC 13616), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus*, (ATCC 14579) and Gram negative strains *Klebsiella pneumoniae* (ATCC 43816), *Escherichia coli* (ATCC 8739), *Proteus vulgaris* (ATCC 13315) were procured from American Type Culture Collection, USA. Methcillin- resistant *Staphylococcus aureus* was purchased from Culture Collections, UK. All bacterial strains stored at -80^{o}C were streaked on Luria-Bertani (LB) agar plates (Hi-media Laboratories, Mumbai, India) and incubated at 37oC for 20 to 24 h. A few isolated colonies were selected from each plate and suspended in 5 ml of LB broth in sterile culture vessel. The vessel was plugged with cotton and incubated with gentle shaking (140 rpm) at 37^{o}C for 20 h.

2.2 Preparation of Bacteria for Bactericidal Assay

Culture (1 ml) of tested organisms were added separately to a 1.9-ml eppendorf tube, and the bacterial sedimentation was achieved by centrifugation at <1000xg for 3 min. The pellet was re-suspended using 1 ml of sterile PBS by gentle aspiration in and out of a transfer pipette. The optical density (OD) of the pellet was determined at 620 nm in spectrophotometer. The OD at 620 of the sample was adjusted approximately to 0.8-0.9 nm by the addition of PBS. Ten microliters of the diluted sample was subjected for serial dilution with PBS, so that these dilutions would produce approximately 1,500 to 2,000 bacteria per 50-ml sample. The ODs of the samples results in 60 to 200 CFU/ mL.

2.3 Preparation of Compound Stocks and Their Dilutions

10, 000 mg of isolated compound was dissolved in one liter of PBS. Further, 1 mL of this solution was diluted in 9 mL of PBS to generate 1000 mg/L stock. This stock was used for serial dilutions to produce the concentrations ranging from 0.1-200 mg/mL. Cefixime is used as positive control (10µg/mL). Solubility of the standards was achieved by adding few drops of saturated NaHCO3. The dilution of the compounds was achieved by dissolving 1 mg of compound in 1L of endotoxin free water. Further 1 mL of this dilution was redissolved in 9 mL of endotoxin free water to produce 10 µg/L concentration and used in the study.

2.4 Determination of Bactericidal Activity

The assay was conducted to assess the bactericidal activities of plant compounds through microtiter plate as described previously elsewhere.[14] The assay reaction mixture consisted of PBS (50mM sodium phosphate, 150 mM NaCl [pH 7.0]), the test compound at various concentrations and the bacterial strains, were prepared in sterile 96-well microtiter plates (Nunc, Inc). The wells are filled with 100µl diluted Physagulin F in PBS and 50 µl of the diluted bacterial strains. The wells were incubated with gentle shaking (140 rpm) at 37oC for various incubation periods (0 (baseline), 2, 4, 8, 12, and 24 h) (time-kill studies) 24 h. For positive and negative controls, a separate microtiter plate was prepared and screened for each incubation time studied (0, 2, 4, 8, 12, and 24 h). Following incubation, a 20-µl aliquot from each well was spotted at the top of a square plate containing Nutrient agar medium. The plate was labeled and tapped gently to facilitate the movement of the liquid. There were approximately 200 cells in the spotted (20-µl) sample. Plates were placed uncovered in biohood until the sample liquid dried (ca. 10 min) and incubated overnight at 37oC. The experiments were performed in duplicate, and CFU for each streak were enumerated after 24 h using a colony counter.

The number of CFU at each dilution of test compounds was compared with the average of positive control value to determine the percentage of bacteria killed per well. The percentage of the bacteria killed was plotted graphically, and the percentage of the test compound resulting decrease in the number of CFU (IC50) was determined.

2.5 Statistical Analysis

Statistical Analysis Data were analyzed using analysis of variance, followed by the Student's t-test, and $p > 0.05$ indicated no significant difference between treatments. The statistical software SAS version 9.0 was employed.

3. RESULTS

3.1 Bactericidal Assay

According to the results obtained, bactericidal efficacy of physagulin F was found high against Methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis*. On the other hand, among Gram negative strains *Escherichia coli* was noticed high susceptibility. *Proteus vulgaris* showed high resistance at all concentrations of the test compound. Bacillus cereus and *Klebsiella pneumoniae* showed moderate susceptibility. The IC_{50} value of the compound against test organism is presented as SEM values in Table 1.

3.2 Effect of Incubation Period on Bactericidal Activity

To find out the sensitivity of the bacterial strains, we have conducted time-kill studies, where the test organisms are incubated at different incubation time periods with test

compound. According to the data of the investigation, we have noticed that for most of the screened bacterial strains, the susceptibility was initiated after 6 hrs. whereas, some of the bacterial strains viz., MRSA, E. *coli*, B. *Subtilis* showed susceptibility within 3 hrs of incubation. The bactericidal activity of physagulin F was found high for first 3-12 hrs and followed by plateau in activity during the next 12-24 hrs. The results are shown in Graph 1.

Table 1: Mean and standard deviation of minimal inhibitory concentration (MIC) values of Physagulin F and Cefixime against testd bacterial strains

	MRSA	B. *Subtilis*	B. *cereus*	E.*coli*	K.*pneumoniae*	P. *vulgaris*
Physagulin F	2.91± 1.5	4.63± 2.0	2.17±1.0	3.16±1.9	2.18±0.9	---
Cefixme	0.91±.0.11	1.16±0.8	1.5±0.9	1.881±0.14	1.08±0.25	---

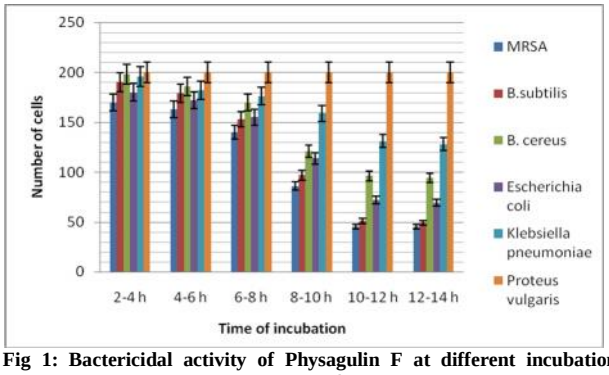

Fig 1: Bactericidal activity of Physagulin F at different incubation periods. Graph represents the significant reduction in number of bacterial strains from 4h-12 h.

4. DISCUSSION

In the previous article we have reported the isolation of Physagulin F (Steroid lactone) from the fruits of *Physalis angulata* fruit extracts. In continuation to the previous investigation, the present study was framed to evaluate the bactericidal efficacy of the physagulin F. Till the date there no findings of bactericidal activity carried out from the crude extracts or isolated compounds of *Physalis angulata*. Therefore, for the first time here we represent bactericidal activity of the compound isolated form *physalis angulata* (Mullaca). Based on the results of the current study, Physagulin F exhibited bactericidal activity against the screened bacterial strains. However, the study noticed that this compound possesses selective inhibition of bacterial growth. This compound showed high activity against Gram positive strains comparing to Gram negative strains. Among the Gram negative strains *Escherichia coli* exhibited high susceptibility nature, *Klebsiella pneumonia* showed moderate susceptible. On the other hand *Proteus vulgaris* was noticed high resistance towards test compound. The selective inhibition property of Physagulin F was correlated with the previously reported antibacterial activity of steroidal lactones.[15-20] The high bactericidal mechanism of this compound is probably accompanied to the absence of OH at 27th Carbon of the compound.[21-22] It is also discussed that 5,6 epoxide system of this compound attribute to high antibacterial action. Most of the compounds isolated from this Physalin *angulata* are Physalins (A, B, D and F) and glycoside Myricetin-3-Oneohesperidoside and these were reported significant anticancer activity on hepatoma, cervix uteri, lung adenocarcinoma, leukemia and epidermoid carcinoma cell lines.[23-25, 5] The fruit extract of *Physalis angulata* demonstrated antibacterial activity on various Gram positive and Gram negative species. However, this work requires proper scientific evaluation and documentary reports to identify and prove as significant antibacterial agents.[26]It has been also reported that the essential oils isolated from *Physalis angulata* exhibited significant antibacterial activities against bacterial strains tested.[27] Physalin B isolated form fruit capsules of Physalin *angulata* also reported high antimicrobial activities.[28] The results from the current study are very encouraging and indicate this compound should be studied more extensively to explore its potential antioxidant and antiinflammatory properties as well.

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