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RESEARCH ARTICLE

IN VITRO ANTIBACTERIAL ACTIVITY OF PHYSAGULIN ISOLATED FROM *PHYSALIS ANGULATA* FRUITS AGAINST PATHOGENIC CLINICALLY IMPORTANT BACTERIA

Swapna Gurrapu and *Estari Mamidala

Infectious Diseases Research Lab, Department of Zoology, Kakatiya University, Warangal – 506009, Telangana State, India

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ABSTRACT

Background: The extracts or infusion of *Physalis angulata* plant fruits were used in the treatment of a wide range of diseases such as asthma, hepatitis, malaria, dermatitis and rheumatism.
Aim: The aim of this study was to test the susceptibility of five human pathogenic bacteria species to Physagulin isolated from the fruits of *Physalis angulata* plant.
Methods: The crude extracts were prepared using different solvents by maceration method and isolation, purification were done by TLC and column chromatography. The isolated Physagulin was screened for antibacterial activity using agar well diffusion and broth micro-dilution assay.
Results: In the present study, the inhibitory action of the Physagulin was found to increase with an increase in concentration against all bacterial strains. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria.
Conclusion: Based on the observations, *Physalis angulata* appears to be a valuable source for antimicrobial properties and helps to produce antimicrobial agents to treat human pathogenic infections.

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INTRODUCTION

The past few decades have experienced an overwhelming increase in global interest on the practice of traditional medicine and its use of medicinal plants to treat illness (Akerele, 1994). Plant-derived preparations and isolated phytochemicals or their model derivatives may be potentially useful to treat infectious diseases, especially in the light of the emergence of drug-resistant microorganisms and the need to produce more efficacious and cost-effective antimicrobial agents (Ncube, 2008). The use of antibiotics has revolutionized the treatment of various bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms (Hart and Karriuri, 1998). This necessitates the need for development of novel antimicrobials (Chopra *et al.,* 1997). One way of preventing antibiotic resistance of pathogenic species is development of new compounds that are not based on existing synthetic antimicrobial agents (Rojas *et al.,* 2006). Plant-derived traditional medicines can be used to treat different diseases as they contain a variety of secondary metabolites to which the bacterial species may not be resistant. The plants have used for centuries by human beings. Traditional medicinal systems are important as a number of important modern pharmaceuticals have been derived from plants used by indigenous people (Swapna Gurrapu and Estari Mamidala *et al.,* 2016). *Physalis angulata*, (a branched annual shrub) is commonly known as gooseberry or sun berry in India, belongs to Solanaceae (Januário *et al.,* 2017). It is majorly distributed in tropical and subtropical regions of the world. The extracts or infusion of this plant is used in the treatment of a wide range of diseases such asasthma, hepatitis, malaria, dermatitis and rheumatism (Lin *e al.,* 1992). Physalins (A, B, D and F) and glycosides such as Myricetin-3-Oneohesperidosideisolated from organic fractions of *Physalis angulata.* Reported significant anticancer activity on various tested cancer cell lines such as HA22T (hepatoma), HeLa (cervix uteri), lung adenocarcinoma, leukemia and epidermoid carcinoma (nasopharynx KB-16) cell lines (Islam *et al.,* 2008). The biological properties of this plant include anticancerous, antimycobacterial, antitumor, hypotensive, immunostimulant, anticoagulant, etc (Januário *et al.,* 2017). This Plant habitat in moist drained and sandy loamy soil and is renowned as effective stimulant for the immune system. The juice of this plant is used for the treatment of jaundice, earache, fever, bladder diseases etc. The fruit and

Corresponding author: Estari Mamidala
Infectious Diseases Research Lab, Department of Zoology, Kakatiya University, Warangal – 506009, Telangana State, India.

aerial parts are extensively used in the treatment of constipation, sores, boils, cuts, intestinal and digestive problems (Sultana *et al.,* 2008). Antibacterial activity was also not studied for isolated phytochemicals present in it. Therefore the objective of this present study is to study the antimicrobial activity of Physagulin isolated from the *Physalis angulata* fruits.

MATERIALS AND METHODS

Plant material

The fully mature *P. angulata* fruits were collected in August-September 2015 from the fields of Karimabad Village in Warangal District of Telangana State, India. The authenticity of the plant was carried out by Prof. V.S. Raju, Taxonomist, Department of Botany, Plant Systematic laboratory, Kakatiya University, Warangal and voucher specimen was deposited in the Herbarium of the Metabolic Disorders Research Lab of the same University.

Preparation of plant extract

The fruits were shade dried and grinded in homogenizer in to coarse powder. The powdered material (250 grams) was extracted by sequential maceration method using n-hexane, chloroform, ethyl acetate, and acetone and methanol (non-polar to polar) solvents. Concentration of extracts was carried out by rot a-vaporization at their boiling points and crude was collected and stored 4°C for further use. The weight of the residual extract was measured and percent yield was calculated.

Extract yield % = W1/W2 X 100;

Where, W1 = Net wt of powder in grams after extraction and W2 = total wt of powder in grams taken for extraction.

Isolation and Purification of Physagulin-F

Thin layer chromatographic studies

Thin layer chromatography (TLC) profile with other physicochemical parameter can be good tool for standardization and validation of plants. TLC profile is simple and effective method for determination of the solvent system. TLC as per conventional one dimensional ascending method using silica gel 60F254,7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample on TLC volume 1µl by using capillary at distance of 1 cm at 1track. Basic solvent system, hexane: ethyl acetate (100:0to 0:100) were used in TLC and the same solvent system was used to run column with methanol extract.

Column Chromatographic Studies

Column chromatography is a purification technique used to isolate compounds from a mixture. In column chromatography, the stationary phase is a solid adsorbent and the mobile phase is a solvent that is added to the top and flows down through the column. Separation is achieved based on the polar and non-polar interactions among the compounds, the solvent, and the solid stationary phase. Usually Silica or Alumina is used as the solid phase in order to setup the column. In this experiment, Silica was used as the solid medium from methanol extract. The column can be prepared using ac olumn chromatography flask. Glass wool was inserted at the bottom of the flask to prevent the silica from escaping the column. The selected mobile phase (hexane: ethyl acetate-100:0 to 0:100) was continuously poured to the top with the aid of a dropper. The bottom outlet of the column was opened, allowing the eluent to flow through the column. As the eluent passed down the column, the compound fraction moved down the column. The separated fraction flowed out of the column where the different elutes were collected in separate test tubes. This was repeated until all the dissolved extract was adsorbed on to the silica gel. The collected elutes were tested in TLC up to single spot appeared.

Structure Elucidation

Based on TLC, the elute is taken further 1HNMR, 13CNMR and Mass spectral studies for structural determination.

Bacterial Cultures

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigellaboydii*, *Staphylococcus aureus* and *Streptococcus faecalis* were obtained from the Department of Microbiology, Kakatiya University, Telangana State, India. All the test strains were maintained on nutrient agar slopes (Hi-Media) and were sub-cultured once in every two-week. These bacteria served as test pathogens for antibacterial activity assay (Rajendra Prasad Gujjeti and Estari Mamidala, 2013).

Antibacterial assays

Agar-well diffusion

The assay was conducted as described by Perez et al. (1990). Briefly, microorganisms from growth on nutrient agar incubated at 37^0C for 18 h were suspended in saline solution 0.85% NaCl. The suspension was used to inoculate 90 mm diameter Petri plates with a sterile non-toxic cotton swab on a wooden applicator. Six millimetres diameter wells were punched in the agar and filled with 50 µl of different concentration (125, 250 and 500 µg/ml) of alkaloids. The dissolution of the extract sample was aided by 1% (v/v) DMSO which did not affect microorganism's growth, according to our control experiments. Commercial antibiotic (Ciprofloxacin) was used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated in air at 37^0C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were conducted twice.

Broth micro dilution assay

Broth micro dilution method was used to determine minimal inhibitory concentrations (MIC) of extract sample against the test microorganisms as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The tests were performed in 96 well-plates. The extracted samples dissolved in 1% DMSO were transferred in plates to obtain a twofold serial dilutions ranging from 10 to 640 µg/ml. Microbial suspensions inoculated in well plates and diluted to have 10^5cfu/ml in each well. The final volumes in wells were 200 µl. MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth after 24 h incubation in air at 37^0C.

Statistical analysis

Values are expressed as mean ± SE. Statistical significance was determined using one-way analysis of variance (ANOVA) and values with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Yield of Crude Extract

The percentage of yield of extract macerated with various solvents are; Hexane 4.92 % (12.3 g), Chloroform 3.38% (8.45 g), Ethyl acetate 1.74% (4.36 g), Acetone 1.22 % (3.06 g), Methanol 5.7 % (14.25 g). Methanol extract of *P. Angulata* obtained the highest percentage yield to comparing to other solvent crude extracts (Table-1).

Isolation of Physagulin-F

Methanol crude extract was filtered and concentrated under reduced pressure to yield a reddish brown residue. This crude extract was fractioned using silica gel (100-200 mesh) column chromatography and the components in crude extract eluted using solvents starting with hexane: ethyl acetate (100:0) and ending with hexane: ethyl acetate (0:100). 12 fractions were yielded and designated as A1 to A12. Fraction obtained from column was monitored according to the variations in composition indicated by the silica gel, 60, F254, TLC. The visualization of spots on the TLC plate's were achieved by exposing TLC plates to iodine vapors after developing hexane and ethyl acetate as solvent system. The TLC pattern of A4 strongly indicates as a single compound.

Characterization and Identification of Physagulin-F

A4 fraction was designated as single compound and characterization was achieved through spectral analysis. Based on the spectral data (Table-2) it is identified that the isolated compound was Physagulin-F. Molecular weight is 544.6332 and IUPAC name is 6-[1-(4,5-dimethyl-6-oxo-2,3-dihydropyran-2-yl)ethyl]-2,16,17-trihydroxy-7,11 dimethyl-12-oxo-5-oxapentacyclooctadec-13-en-3-ylacetate (Steroid Lactones). Mass spectra and 1HNMR of Physagulin-F isolated from fruit of *P. angulata* were shown in Figure-1 and Figure-2. The structure of the isolated compound was shown in Figure-3.

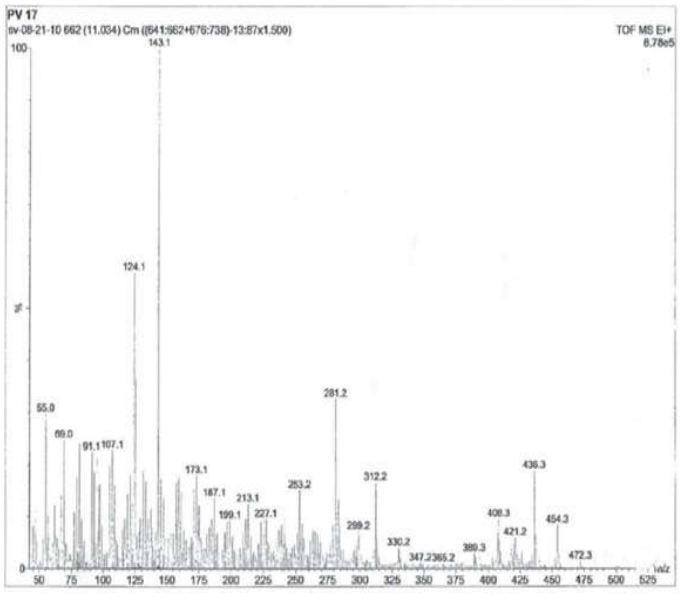

Figure 1. Mass spectra of Physagulin-F

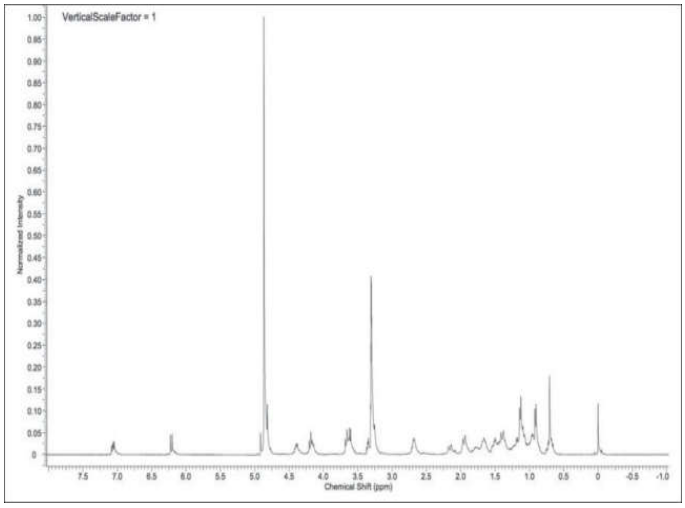

Figure 2. [1]HNMR spectra of physagulin V. The spectrum was obtained at 400 MHz in MeOD at 27 °C with an acquisition time of 3.9715 s

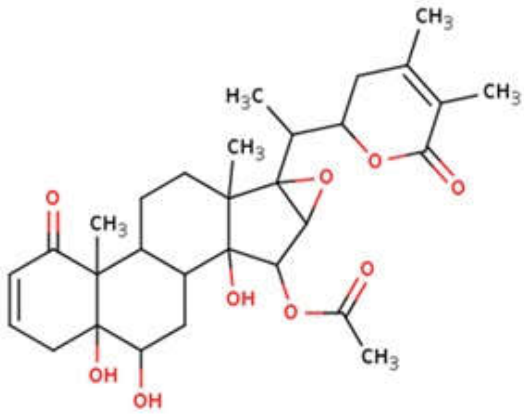

Figure 3. Molecular structure of Physagulin-F isolated form *Physalis angulata*

Antibacterial activity

The antibacterial activity of isolated Physagulin compound was determined by using agar well diffusing method and broth micro dilution assay. The results in Table-3 and Table-4 show that the isolated compound has good antibacterial activity against selected human pathogenic bacteria. In the present study, the inhibitory action of the Physagulin was found to increase with an increase in concentration against all bacterial strains. The tested bacterial strains showed different patterns of inhibition. This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity (Rajendra Prasad Gujjeti and Estari Mamidala, 2013). The broad spectrum of antibacterial activity was reported for *Physalis angulata* (Ogunlana, 1975). The isolated Physagulin compound at a concentration of 500 µg/disc showed maximum inhibition against *S. aureus*(14.5 mm), followed by *E. coli* (12.7 mm), *S. Boydii* (11.2 mm), *S. Faescalis* (10.5 mm) and *P. Aeruginosa* (7.8 mm) by agar well diffusion method. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria. Physagulin showed maximum activity against gram-negative bacteria and showed the highest inhibition zones against *P. aeruginosa* and *E. coli*. This study confirms the Physagulin compound may be responsible for the antibacterial activity against various bacterial strains.

Table 1. Percentage yield of crude extract of four plants

Plant	Solvent extract	Weight in grams	% Yield
P. angulata (Fruit)	n-Hexane	12.3	4.92 %
	Chloroform	8.45	3.38 %
	Ethyl acetate	4.36	1.74 %
	Acetone	3.06	1.22 %
	Methanol	14.25	5.7 %

Table 2. ^{1}HNMR and ^{13}C-NMR data of the Physagulin-F isolated from fruit of *Physalis*

^{1}H NMR spetral data:
6.14dd (9.9,2.2), 6.69 ddd (9.9,5.1,2.2), 3.74 dt (22.2,2.2), 2.38 dd (22.2,5.1), 4.15 br s, 2.59 brdt (12.5,2.8) 2.38 br td (12.5,2.7), 2.51 br td (12.8, 2.8), 3.18 br td (12.8.2.6) 2.67 br td (13.0, 2.6), 1.42 m, 2.01 br t (13.0), 1.71 br d (12.8), 5.65 s,3.73 s,4.51 ddd (12.8,5.5,3.7), 2.33 br d(16.0), 2.13 brdd (16.0, 3.0), 1.91 s, 1.74 s,-OAc,2.25 s.
^{13}CNMR spectral data:
C-1(203.9),C-2(128.8) C-3(141.2) ,C-4(36.2),C-5(76.3),C-6(74.56),C-7(28.3),C-8(35.0),C-9(35.3),C-10(51.9),C-11(21.8), C12 (32.6), C13 (46.6), C14 (81.7), C15 (76.8),C16 (59.2),C17 (23.1), C18 (15.9), C19 (14.9), C20 (33.1), C21 (13.52), C22 (76.7), C23 (32.4), C24(149.1), C25(121.9), C26 (160.3), C27(12.4), C28(20.5)-OAC

Table 3. The zone of inhibition produced by Physagulin compound isolated from *Physalis angulata* fruits.

Bacteria Strains	Inhibition zone (mm)				
	125 µg/ml	250 µg/ml	500 µg/ml	Standard (Ciprofloxacin, 25 µg/disc)	Control (DMSO)
Escherichia coli	9.0 ± 0.01	9.4 ±1.97	12.7 ±1.99*	18.3 ±1.93	0
Pseudomonas aeruginosa	0.00 ± 0.00	7.0 ± 1.99	7.8 ± 1.95	8.5 ±1.94	0
Shigellaboydii	8.2 ± 1.94	8.5 ± 0.00	11.2 ±1.98*	9.2 ±1.93	0
Staphylococcus aureus	10.5 ± 1.98	11.3 ±1.99	14.5 ±1.98*	21.5 ±1.88	0
Streptococcus faecalis	7.7 ±1.93	8.0 ±1.98	10.5 ±1.94*	18.6 ±1.87	0

The varying concentrations between 10 to 640 µg/ml of the isolated Physagulin compound of *Physalis angulata* were tested in order to determine their MICs by broth micro dilution assay. The MICs of the isolated Physagulin compound against the five tested bacterial is presented in Table-4. The lowest MICs were obtained in the Physagulin compound having 40 µg/ml against *E. coli,* 55 µg/ml against *S. aureus*, 59µg/ml against *S. boydii*, 80 µg/ml against *P. Aeruginosa* and 87 µg/ml against *S. faescalis*. The MIC ranged from 10 to 640 µg/ml for all studied microorganisms while for ciprofloxacin it ranged from 0.1 to 10 µg/ml. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria. In this study, this antimicrobial activity may be due to the presence of (OH) group in the structure isolated Physagulin which increased the activity to inhibit the bacterial growth by changing the nature of cell protein (denaturation), thus increasing the permeability of cell membranes (Caceres *et al.,* 1995), either by increasing the permeability of the cell membrane of the bacteria. The cell membrane causes loss or leakage of the contents of a cell of bacteria to the outside or through a direct link membrane of cell bacteria, causing the demise of polar membrane of bacteria, which leads to the death of a cell bacteria gradually (Gurrapu *et al.,* 2017).

Conclusion

The result of the present study showed that the isolated Physagulin compound of the fruits of *Physalis angulata were effective* against the bacterial species tested. The extracts or infusion of this plant is used in the treatment of a wide range of diseases such asasthma, hepatitis, malaria, dermatitis and rheumatism. This investigation has opened up the possibility of the use of this plant for formulating a drug for human consumption possibly for the treatment of bacterial infections. These findings support the traditional knowledge of local users about their selection of this plant sample as antimicrobial agents and it is a preliminary scientific validation for the use of this plant for antibacterial activity. The results of the present study also support the medicinal usage of the Physagulin isolated from the fruits of *Physalis angulata* can be used as antimicrobial agents in new drugs for therapy and can be subjected to identification and isolation of the therapeutic antimicrobials and undergo further pharmacological screening that can be used as sources for new drugs.

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