Haya: The Saudi Journal of Life Sciences (SJLS)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2415-623X (Print)
ISSN 2415-6221 (Online)

In vitro Antibacterial Activity of Acalyphin Compound Isolated from Leaves of *Acalypha indica* Against Human Pathogenic Bacteria

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Original Research Article

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Article History
Received: 18.12.2017
Accepted: 27.12.2017
Published: 30.01.2018

DOI:
10.21276/haya.2018.3.1.1

Abstract: The aim of the present study was to investigate the antibacterial properties of Acalypha indica. Number of resistant antibiotics is increasing and antibacterial activity testing is one of the steps to find treatment of diseases. Acalypha indica is well known medicinal plant and it is a wild plant. It was used as infection treatment for generation to treat several diseases such as asthma, and pneumonia.The susceptibility of five human pathogenic bacterial species to Acalyphi**n i**solated from the leaves of *Acalypha indica* plant was screened using the agar well diffusion and broth micro-dilution assay. The purity of Acalyphin isolated was checked by TLC and column chromatography and total Acalyphin were quantified. In the present study, the inhibitory action of the Acalyphin 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. From the above investigation the experimental *Acalypha indica* plant may solve the multidrug resistant bacteria problem and further higher studies is need for qualitative study for the present investigation.

Keywords: *Acalypha indica,* Acalyphin**,** Euphorbiaceae, human pathogenic bacteria, Diseases, Agar well diffusion, Antibacterial activity.

INTRODUCTION

Many of the plants used today were recognised to the people of earliest cultures throughout the world and were highly measured their preservative and medicinal powers.

Scientific trials on the antimicrobial possessions of plants and their constituents have been recognised in the late 19th century [1]. India has a gorgeous flora that is extensively dispersed throughout the country. Herbal drugs have been the basis of management and cure for numerous ailments and physiological surroundings in traditional methods accomplished such as Ayurveda, Unani and Siddha. Therapeutic constituents from plants play an significant role in conventional as well as western treatment. Plant derived medications have been a part of the progression of human, health care for thousands of years. Plant based medications were frequently used in India and China [2]. At the same time, native people of the rest of the world were also applying plants for curative diseases. Most of the plant resources have been studied on bacteria. There are a huge number of investigators in diverse parts of the word [3-5]. A huge number of traditional normal products has interest grown up [4]. Traditional therapeutic systems are significant as a number of essential modern treatments have been consequential from plants used by indigenous people [7].

Acalypha indica L. (family: *Euphorbiaceous*) is a weed widely dispersed throughout the plants of India. It has been described to be useful in handling pneumoniae, asthma, rheumatism and several other ailments [8]. The parched leaves of *Acalypha indica* was prepared into a compress to treat bedsores and wounds and the juice of *Acalypha indica* is added to oil or lime and used to treat diverse skin disorders. The leaves of *Acalypha grandis* have also been reported to have contraceptive activity [9]. Several biochemical [10] and biological [11] innovations have been approved out on this plant. Therefore the objective of this present study is to study the antimicrobial activity of Acalyphin isolated from the leaves of *Acalypha indica* plant extract against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Plant Collection

Based on our previous research work [12] and its traditional medicinal use the *Acalypha indica* plant was selected for this study. Plant leaves were collected from the rural areas of Jannaram forest, Adilabad

district, Telangana, India in the month of October 2016. The plant voucher specimens Identification was done with the help of Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal and the same was deposited at Infectious Diseases &Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal.

Extraction and isolation of Acalyphin

About 500 g of air-dried powdered leaves of *A. indica* were extracted with petroleum ether (40–60°C) by maceration (1.2 L × 4) and the extract was evaporated under reduced pressure at 45°C and the residue dissolved in hot distilled water (400 ml) and left in the refrigerator overnight and filtered the precipitated matters. The filtrate was partitioned with chloroform (500 ml × 2) and *n*-butanol (600 ml × 4). The butanol extract was concentrated and subjected to column chromatography. Elution was started with distilled water and decreasing the polarity in 10% with methanol to 100% methanol. The fractions were collected by monitoring on thin layer chromatography (TLC) using solvent 1 and/or solvent 2 as developing solvents. The fraction eluted with aqueous methanol (40% and 50%) was found to active and this fraction was subjected again to column chromatography using 30% methanol for elution and increasing methanol to 70% as mentioned above. Based on the NMR and MS studies, the isolated compound (10 mg) was identified as acalyphine.

Bacterial Cultures

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Staphylococcus aurous* 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[12].

Antibacterial assays

Agar-well diffusion

The assay was conducted as described by Perez *et al*. [13]. 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 alkaloids was aided by 1% (v/v) DMSO which did not affect microorganisms' 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 alkaloids against the test microorganisms as recommended by the National Committee for Clinical Laboratory Standards [14]. The tests were performed in 96 well-plates. Alkaloids dissolved in 1% DMSO were transferred in plates to obtain a twofold serial dilutions ranging from 10 to 640 µg/ml. Then plates were inoculated with microbial suspensions diluted to have 10^5 cfu/ml in each well. The final volumes in wells were 200 µl. After 24 h incubation in air at 37^0C, MIC was recorded as a lowest extract concentration demonstrating no visible growth in the broth.

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 DISCUSSIONS

Antibacterial study

The antibacterial activity of isolated Acalyphin compound was determined by using agar well diffusing methods. The results in Table-1 show that the isolated compound has good antibacterial activity against selected human pathogenic bacteria. In the present study, the inhibitory action of the Acalyphin 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 [15]. The broad spectrum of antibacterial activity was reported for *Physalis angulata* **[**7].

The isolated Acalyphin compound at a concentration of 500 µg/disc showed maximum inhibition against *S. aureus* (17 mm), followed by *E. coli* (15.2mm), *S. boydii* (13.7 mm), *S. faescalis* (13 mm) and *P. aeruginosa*.(10.3 mm) by broth dilution method. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria.Leaves of *Acalypha indica* plant methanolic extract showed maximum activity against gram-negative bacteria and showed the highest inhibition zones against *P. aeruginosa* and *E. coli*. This study confirms the presence of the Acalyphin compounds may be responsible for the antibacterial activity against various bacterial strains.

Table-1: The zone of inhibition produced by Acalyphin compounds isolated from *Acalypha indica* leaves

Bacteria Strains	Inhibition zone				
	125µg/ml	250µg/ml	500µg/ml	Standard (Ciprofloxacin, 25 µg/disc)	Control (DMSO)
Escherichia coli	11.5 ±0.53	11.9 ±0.53	15.2±0.51*	20.5± 0.57	0
Pseudomonas aeruginosa	0.00 ± 0.5	9.5 ± 0.51	10.3 ± 0.55	11 ± 0.56	0
Shigella boydii	10.7 ±1.56	11 ± 0.5	13.7±0.52*	11.7 ± 0.57	0
Staphylococcus aureus	13 ± 0.52	13.8 ±0.51	17 ± 0.52*	24± 0.62	0
Streptococcus faecalis	10.2 ±1.57	10.5 ±0.52	13 ± 0.56*	21.1± 0.63	0

Values are expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) followed by Dennett's *t* test; $*P < 0.05$

The varying concentrations between 10 to 640 ug/ml of the isolated Acalyphin compound of *Acalypa* indica were tested in order to determine their MICs. The MICs of the isolated Acalyphin compound against the five tested bacterial is presented in Table-2. The lowest MICs were obtained in the Acalyphin compound having 42.5 µg/ml against *E. coli*, 57.5 µg/ml against *S. aureus*, 61.5 µg/ml against *S. boydii*, 82.5 ug/ml against *P. aeruginosa* and 89.5 µg/ml against *S. faescalis*. The MIC ranged from 10 to 640 µg/ml for all studied microorganisms while for ciprofloxacin it ranged from 1 to 10 µg/ml. These Acalyphin known to be biologically active as well as showing antimicrobial activities. 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 Acalyphin which improved the activity to obstruct the bacterial growth by changing the nature of cell protein (denaturation), thus increasing the permeability of cell membranes [16], either by increasing the permeability of the cell membrane of the bacteria. The cell membrane causes injury or leakage of the insides 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 cell bacteria gradually [17].

Table-2: Antibacterial activity of Acalyphin compound isolated from Acalypha indica leaves *against* selected human pathogenic bacteria by minimum inhibitory concentration (MIC) method

Bacterial strains	MICs (µg/ml) of isolated alkaloid compound	Standard (Ciprofloxacin)	Control (DMSO)
Escherichia coli	42.5	1.5	0
Pseudomonas aeruginosa	82.5	1.3	0
Shigella boydii	61.5	1.5	0
Staphylococcus aureus	57.5	1.5	0
Streptococcus faecalis	89.5	1.8	0

CONCLUSION

The result of the present study showed that the isolated Acalyphin compound of the fruits of Acalypha indica were effective against the bacterial species tested. Traditionally, root of Acalypha indica used as infection treatment for generation to treat several diseases such as asthma, and pneumonia. It is possible to use this plant for human consumption to treat 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. To ensure good results for scientific studies on medicinal plants containing traditional arguments of results. These plants serve useful resources for the new antimicrobial agent.

ACKNOWLEDGEMENT

The authors are thankful to Department of Microbiology, Kakatiya University, and Warangal for providing test pathogens for carrying this work.

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