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Antimicrobial Compound Isolated from the Plant Pothos scandens L.

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Abstract

Pothos scandens L. is a medicinal plant found in North East India with various ethno-medicinal activities. A compound was isolated from the bark of *P. scandens* and the structure was characterized as Octyl isonicotinate (**1**) by extensive NMR, FT-IR and mass analysis. Compound **1** exhibited significant antimicrobial activity against *Escherichia coli* and *Candida albicans* by Agar well diffusion method with zone of inhibition value of 9.16±0.28 mm and 12.5±0.51 mm, respectively compared with standards Amikacin (19.33±0.57 mm) and Fluconazole (15.9±0.12 mm). The minimum inhibitory concentration (MIC) values were found 12.30 µg/mL and 6.50 µg/mL respectively as compared to Amikacin (4.37 µg/mL) and Fluconazole (7.5µg/mL).

Keywords: *Pothos scandens*; Octyl isonicotinate; antimicrobial.

1. Introduction

Medicinal plants have been utilized as a source of medicine to treat numerous diseases and disorders since time immemorial [1-3]. The most of world's population in developing countries rely on medicinal plants for their primary health care [4-5]. India has a rich tradition of using medicinal plants for the treatment of various infectious diseases, inflammations, injuries and other diseases [6]. Thereby, for the treatment of bacterial infections, in recent times, plant derived natural products have received the tremendous attention of researchers due to their diverse pharmacological properties.

Antibiotic resistance becomes an ever-increasing therapeutic problem all over the world by the emergence of multi-drug resistant pathogens, which are causative agent of various human diseases like, hemolytic uremic syndrome in humans (HUS), urinary infections, opportunistic oral and genital infections, nail infection, candidiasis, pneumonia, endocarditis, toxic shock syndrome, bacteremia, and sepsis [7-13]. In recent years, the systematic screening of the biological interaction between microorganisms and plant products has been recognized as a valuable source of several compounds able to control the survival of pathogen microorganisms.

Pothos scandens L. is a species of *Pothos* genus belongs to the family Araceae that is used in folk medicine in Northeast India [14]. *P. scandens* is an epiphyte with climbing and rooting branches. Its leaves are (5-10) x (1-5) cm, distichous, ovate or ovate-lanceolate, acute or acumidate, obliquely linear with broad flat truncate petiole, sheathing at the base, having a bright green colour. The fruits or berries are 1.3-1.7 cm long and oblong and when ripe they become scarlet. Seeds are 3-6 mm in diameter,

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ellipsoid to compressed-spherical with smooth testa and lacking albumen [15-16].

The species contains several secondary metabolites and showed diverse bioactivities such as antimicrobial, antioxidant, antipyretic, anticariogenic, anticancer, thrombolytic etc. [17-30]. In this paper, we have shown the isolation of a compound from *P. scandens* by chromatographic techniques and structure elucidation by various spectroscopic methods. The antimicrobial activity study against *Escherichia coli* and *Candida albicans* was done by agar well diffusion method.

2. Experimental

2.1. Materials and methods

^{1}H- and ^{13}C-NMR were recorded with a Bruker AVANCE DPX 500 MHz NMR spectrometer, Switzerland with internal reference as tetramethylsilane (TMS). Chemical shifts (δ) value are reported in ppm and coupling constant (*J*) in Hz. Melting point was performed using BUCHI M-560 capillary melting point apparatus and is uncorrected. A high resolution mass spectrum (HR-ESI-MS) was recorded in a Waters XEVO G2-Xs QT instrument. A FT-IR spectrum in Elmer FT-IR 2000 spectrometer on a thin film using chloroform was recorded. Silica gel 60-120 mesh was used for column chromatography. Thin layer chromatography (TLC) experiments were performed using pre-coated silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany). Fractionated solvents from column chromatography were evaporated to dryness under reduced pressure by Rotavapor; BUCHI, Labortechnik AG, Flawil, Switzerland. The microorganisms *Escherichia coli* (ATCC®11229TM) and *Candida albicans* (ATCC®90028TM) for antimicrobial assay were purchased from HiMedia.

2.2. Plant material

The bark of *P. scandens* was collected from Golaghat district of Assam, India in 2017. The collected plant materials were further crushed and shade dried, powdered and used for extraction.

2.3. Extraction and isolation

A known amount of powdered bark (800 g) was soaked in hexane (5 L) for 24 h with intermittent shaking. The liquid mixture was filtered and the filtrates were concentrated under reduced pressure using Rotavapor at 46°C. The resulting crude extract was stored at (-) 20°C in a deep freezer to obtain 20 g extract (PSH).

Approximately 500 g of silica gel with mesh size 60-120 was mixed with 1000 mL of *n*-hexane to make silica gel slurry which was then packed into a column. About 3 g of the crude hexane extract was loaded onto the column and a stepwise in gradient solvent system started with 100% *n*-hexane followed by gradually increased polarity using EtOAc (*n*-Hexane/EtOAc ratios 10:1, 7:1, 3:1, 1:1, 1:2, 1:7 and 1:9) to give 116 eluents. All the collected eluents were checked by thin layer chromatography (TLC) analysis and the eluents having similar R_f were combined together and found 8 different fractions. The solvent was evaporated under reduced pressure by rotary evaporator at 48°C. The fraction eluted by *n*-Hexane/EtOAc of 10:1 was purified by preparative TLC with similar R_f to obtain **1**.

Compound 1: White powder (120 mg), R_f 0.6 (*n*-hexane: EtOAc, 10:1), m.p. 18.2-19.3°C. FT-IR (KBr, $CHCl_3$, cm^{-1}): 3416.8, 2923.3, 2852.2, 2100, 1638.6, 1456.7, 1259.9, 1219.7, 1023.4, 722.2, 666.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.64 (dd, *J* = 5.5 & 3 Hz, 2H, H-2′ & 6′), 7.48 (dd, *J* = 5.5 & 3.5Hz, 2H, H-3′& 5′), 4.15 (qd, *J* = 11, 6Hz, 2H, H-1), 1.61 (m, 2H, H-2), 1.29-1.18 (10 H, overlapping signals of H-3, H-4, H-5, H-6 & H-7), 0.84 (t, *J* = 8Hz, 3H, H-8). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 167.76, 132.23, 130.87, 128.69, 68.01, 38.51, 30.18, 28.78, 23.54, 22.90, 14.03, 10.87. HR-MS (+ESI) for $C_{14}H_{21}NO_2$ $[M+H]^+$ at m/z 236.1729 (Calcd for $C_{14}H_{22}NO_2$: 236.1606). Elemental analysis: Found C, 70.87%; H, 8.98%; N, 5.92%; $C_{14}H_{21}NO_2$ requires C, 71.46%; H, 9.00%; N, 5.95%.

2.4. Antimicrobial assay

Test samples were prepared in DMSO and antimicrobial activity was evaluated by well diffusion assay [31]. Mueller hinton agar medium used for the bacterial activity and Potato dextrose agar medium used for the fungal activity. Six mm wells were bored in petriplates which were previously inoculated with respective microorganisms. Final inoculum concentration used for the assay was approximately 1×10^{8} CFU/mL. 100µl of stock solution was then added to the wells and the plates were incubated at optimum temperature. Here Amikacin and fluconazole were used as standard antibiotics with final concentration of 50µg/mL and 60µg/mL, respectively. The zone of inhibition was evaluated

against test organisms and values were expressed as Mean ± SD.

Minimum inhibitory concentration (MIC) was determined by resazurin reduction assay in 96 well microtitre plates (Nunc™, Thermo Fisher Scientific Inc). Resazurin is an indicator that becomes pink, fluorescent when reduced to resorufin and becomes colourless when further reduced. At first 100 µL of sterile broth was added to all 96 wells and test samples were serially diluted. Then 20µl of microbial suspension was mixed to each well to achieve a concentration of 5×10^5 CFU/mL. Finally, 20µl of resazurin indicator was added to each well. Plates were incubated at 30˚C (fungus) and 37˚C (bacteria) for 18-48 h and colour change was then assessed visually. In each plate one column is for sterility check (broth+test sample+resazurin), one is for negative control (broth+inoculum+resazurin), one is for positive control (broth+inoculums+antibiotic) and in rest of the columns test sample was added in duplicates. The plates were observed visually for the colour change of the indicator. The colour change from purple to pink or colourless indicates growth of microbes. MIC of test sample was determined as the lowest concentration of the sample.

3. Results and Discussions

Octyl isonicotinate (**1)** was obtained as a white amorphous powder (120 mg) with molecular formula $C_{14}H_{21}NO_2$, by elemental analysis and positive ion high resolution mass spectrometry, HRMS (+ESI) (m/z 236.1729). In FT-IR spectrum, the absorption band observed at 2923.3 and 2852.2 cm^{-1} due to the presence of CH, CH_2 groups. The IR spectrum displayed aromatic system with C=N (2100.6 cm^{-1}) and –COO– ester groups (1638.6 cm^{-1}). The ^{1}H NMR spectrum, two doublets of doublet at δ_H 7.64 with J = 5.5 & 3Hz and 7.48 with J = 5.5 & 3.5Hz, each integrating to two protons attached to a pyridyl system in the molecule. The quartet of doublet at δ_H 4.15 ppm integrating to two protons confirmed the presence of the ester group. A three proton singlet at δ_H 0.84 ppm were assigned to methyl group. Further, the ^{13}C NMR spectrum of the molecule indicated the presence of four aromatic CH (δ_C 132.23 and 128.69 ppm), one methyl group (14.03 ppm), seven methylenes (δ_C 22.90, 23.54, 28.78, 30.18, 38.51, 68.01 ppm) in addition to quarternary aromatic carbon (δ_C 130.87 ppm) and carbonyl carbon (δ_C 167.76 ppm) (Figure 1).

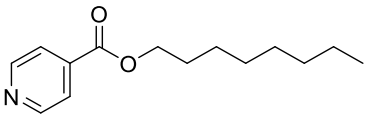

Figure 1 Structure of Compound **1**

The antimicrobial activity of *P. scandens* is given in Table 1. Compound **1** was found highly active against the tested microorganisms with zone of inhibition value of 9.16±0.28 mm and 12.5±0.51 mm against *E. coli* and *C. albicans*, respectively compared with Amikacin (zone of inhibition value of 19.33±0.57 mm) and Fluconazole (zone of inhibition value of 15.9± 0.11 mm). The MIC values of Compound **1** are 12.30 and 6.50 µg/mL against *E. coli* and *C. albicans* respectively, whereas the MIC values of standards Amikacin and Fluconazole are 4.37 and 7.5 µg/mL respectively (Figure 2).

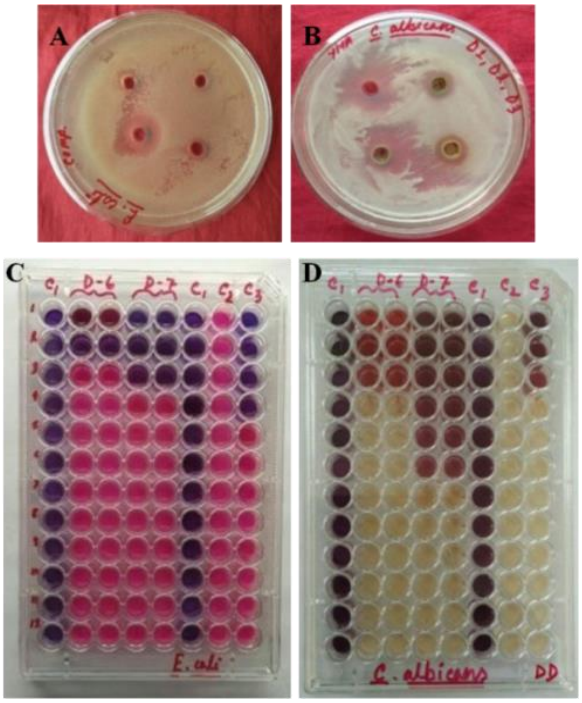

Figure 2 Antimicrobial activity of Compound **1** against (A) *E. coli* with Amikacin +ve control, (B) *C. albicans* with Fluconazole +ve control; (C-D) MIC of Compound 1 against *E. coli and C. albicans*.

Due to the emergence of multi-drug resistant pathogens, antibiotic resistance causes various human diseases. In this study, the Octyl isonicotinate, isolated from *P. scandens* showed significant antimicrobial activity against *E. coli* and *C. albicans*. Since, Amikacin and Fluconazole have considerable side effects, thus compound **1** can be a possible alternative to these two drugs. Our study suggests

that this compound may be used as an efficient antimicrobial drug in the future. Though we have studied the *in vitro* antimicrobial activity of the isolated compound, we can study further for *in vivo* evaluation of microbial profile, which will strengthen our methodology to the development of an effective as well as safer herbal drug formulation lead.

Table 1 Antimicrobial activity of compound **1**

Samples	ZOI against microbes (mean ± SD) (mm)		MIC (µg/mL)	
	E. coli	*C. albicans*	*E. coli*	*C. albicans*
Compound 1	9.16 ± 0.28	12.5 ± 0.51	12.30	6.50
Amikacin	19.33 ± 0.57	ND	4.37	ND
Fluconazole	ND	15.9 ± 0.11	ND	7.5

[a]ND: Not Determined, –: Activity not found,
ZOI= Zone of inhibition in mm, MIC = Minimum inhibitory concentration

4. Conclusions

We have studied *P. scandens*, a medicinal plant in North East India. A compound was isolated from the crude hexane extract and structures were determined by extensive use of ^{1}H and ^{13}C NMR spectroscopic and HRMS methods. Compound **1** exhibited significant antimicrobial activity against *E. coli* and *C. albicans* with MIC 12.30 µg/mL and 6.50 µg/mL respectively as compared to standard marketed drug Amikacin (4.37 µg/mL) and Fluconazole (7.5 µg/mL). Compound **1** was isolated for the first time from any natural source with antimicrobial activity.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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7. Acknowledgments

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