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ORIGINAL ARTICLE

Antimicrobial activities of Conyzolide and Conyzoflavone from *Conyza canadensis*

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Abstract

Antibacterial and antifungal activities of the two isolated compounds from *Conyza canadensis* have been reported in the current study. The two isolated compounds i.e. Conyzolide (**1**) and Conyzoflavone (**2**) were tested against six bacterial and five fungal strains, employing hole diffusion and macrodilution methods. Both the compounds showed significant activities against the tested pathogens with special reference to *E. coli*, *P. aeruginosa*, *S. aureus*, *Trichophytom longifusus*, *C. albicans*, and *C. glaberata*. Conyzolide revealed comparatively better antibacterial activity against *E. coli* (minimum inhibitory concentration (MIC): 25 µg/mL) in comparison to Conyzoflavone. However, in case of antifungal activities, Conyzoflavone exhibited superior antifungal activity against *C. albicans* (MIC: 10 µg/mL) as compared to Conyzolide.

Keywords: *Conyza canadensis*, antibacterial, antifungal, Conyzolide, Conyzoflavone

Introduction

Worldwide infectious diseases are one of the major causes of deaths and responsible for approximately one-half of all the deaths in tropical countries[1]. New, effective and safe therapeutic agents and strategies are demanding issues to cope with the infectious diseases. Low-income people especially from small isolated villages and native communities in developing countries use folk medicine for the treatment of common infections. These medicinal plants are ingested as decoctions, teas and juice preparations to treat respiratory infections or as a poultice and applied directly on the infected wounds or burns[2,3]. So far extensive work has been done and still going on for the search of bioactive compounds to treat microbial infections as more effective and safer therapeutic agents.

Genus *Conyza*, belonging to the family Asteraceae, comprises about 50 species, which are found in the tropical and warm regions. The plant *Conyza canadensis* is commonly known as Canada fleabane, bitterweed and horseweed etc[4]. It is found in all warm countries, but is presumed to be of American origin and spread on the rest of the globe because of its import from that continent[5]. *Conyza canadensis* (syn. *Erigeron canadensis*), (*Asteraceae*) is an annual herb that is distributed throughout world including Pakistan. *Conyza canadensis* is used locally as traditional vegetable and sweetening agent in northern areas of Pakistan and rest of the countries[4]. This plant is reported to be astringent, stimulant, hemostatic, and diuretic, also used in diarrhea, dysentery uterine hemorrhages, dropsy, gravel, cystitis, calculus, bronchial catarrh, and hemoptysis in folk medicine[6]. In Africa, it is used for the treatment of ringworm and eczema. This plant is traditionally used in folk medicines in the northern areas of Pakistan for the treatment of various pathological conditions including its use in acute pain, inflammation, fever and especially the microbial infections including urinary infections, respiratory tract infections, diarrhea and dysentery. Current study was designed

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to identify the potential bioactive compounds exhibiting significant antibacterial and antifungal activities possibly responsible for its folk use in infectious diseases.

Materials and methods

Plant material

The plant specie *Conyza canadensis* was selected based on its ethno-pharmacological knowledge. The fresh whole plant material was collected from village Madyan, District Swat, NWFP, Pakistan. The plant material was identified by Professor Dr. Abdul Rashid Department of Botany University of Peshawar, Peshawar Pakistan.

Extraction and isolation

General extraction procedures were adopted as reported earlier[7–12]. The whole plant material dried under shade was chopped and pulverized into fine powder. A quantity of 4.0 kg of dried powder was macerated with 80% ethanol three times at room temperature. The resulting ethanolic extract (349.13 g) was evaporated under vacuum by rotary evaporator at 50°C that afforded a gummy residue. The crude extract (340 g) was suspended in water and fractionated successively with n-hexane, chloroform and ethyl acetate, followed by leaving a residual water-soluble fraction. Each fraction was then concentrated using rotary evaporator at 50°C to yield n-hexane fraction (60.7 g, 17.85%), chloroform fraction (79.1 g, 23.26%) and ethyl acetate fraction (57.6 g, 16.94%), the remaining was water fraction (142.3 g, 41.85%).

Chloroform fraction (75 g) was subjected to column chromatography (CC) using silica gel with elution started from n-Hexane followed by increasing its polarity by using n-Hexane-chloroform gradients. Finally the column exhausted by gradual increased in polarity of the mobile phase upto 2% methanol chloroform gradient. Chloroform fraction afforded nine subtractions (C1 to C9). Subfraction C-6 (316 mg), was rechromatographed over flash silica and upon elution with n-hexane-chloroform (6:4) gradients resulted in the isolation of compound **1** (named as Conyzolide, 138 mg). Similarly ethyl acetate fractions was loaded on silica gel for CC, initially using 100% n-Hexane followed by n-Hexane-ethyl acetate gradient with increasing polarity upto 5% methanol-ethylacetate gradient. Finally, the ethyl acetate fraction was chromatographed further into 13 subfrcations (E1-E13). Subfraction E-7 (479 mg) was further purified through CC using 80% ethyl acetate/n-Hexane gradient which yielded another compound **2** (named Conyzoflavone, 223 mg). Structures of both the compounds were confirmed from mass and NMR spectral data reported in the literature[13,14] however names were assigned for the first time.

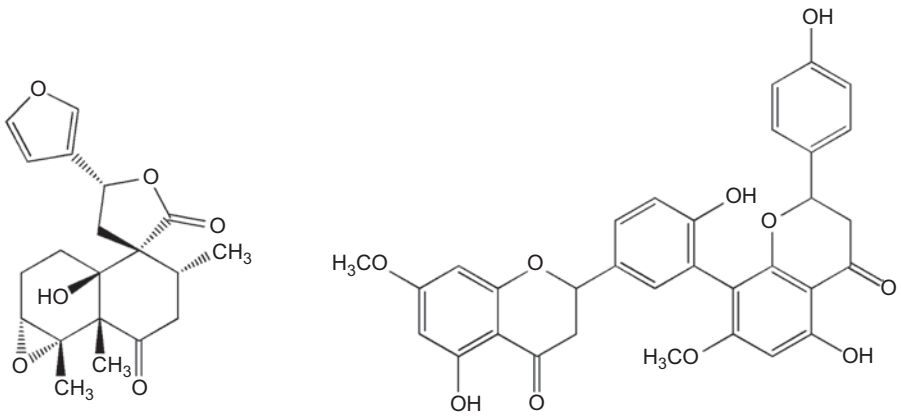
Antimicrobial studies

Fungal and bacterial strains

Tests were performed on six fungal and five bacterial strains. Bacterial strains were *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. flexeneri (clinical isolate), S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 *and S. typhi* ATCC 19430. Fungal strains include *T. longifusus (*clinical isolate*), C. albicans* ATCC 2091, *A. flavus* ATCC 32611, *F. solani* 11712 *and C. glaberata* ATCC 90030. They were maintained on an agar slant at 4°C. The strains were activated at 37°C for 24 h on nutrient agar or Sabouraud glucose agar, respectively for bacteria and fungi, prior to any screening.

Agar well diffusion method

Antimicrobial tests were carried out as reported earlier [Nisar et al., 2008b; Nisar et al., 2009; Jan et al., 2009), by the hole-diffusion method using a cell suspension of about 1.5 × 106 CFU/mL obtained following Macfarland turbidity standard no. 0.5. The concentration of the suspension was standardized by adjusting the optical density to 0.1 at 600 nm (Shimadzu, UV-VIS Spectrophotometer). Holes of 6 mm diameter were then made on the Mueller–Hinton agar plate (8 mm thick) and filled with 150 µL of methanolic extract, fractions or standard drug(s). The inoculated plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of growth inhibition around the



Conyzolide (1) Conyzoflavone (2)

Figure 1. Structures of the isolated compounds.

hole. The assay was repeated three times and the mean diameter was recorded. Streptomycin, miconazole and amphotericin B were used as standard antibiotics for comparison with extracts and fractions.

Determination of MIC by macro dilution method

Compounds were dissolved in dimethylsulfoxide (DMSO) and serially diluted with sterile water in microplates in a laminar flow cabinet. The same volume of an actively growing culture of the test bacteria was added to the different wells and cultures were grown overnight in 100% relative humidity at 37°C. Next morning, tetrazolium violet was added to all the wells and the growth was indicated by a violet color of the culture. The lowest concentration of the test solution that led to inhibition of growth was taken as the minimum inhibitory concentration (MIC). The negative control i.e. DMSO had no influence on the growth even at the highest concentration used. Imipenem, amphotericin B and miconazole were used as controls for comparison.

Results

Antibacterial activity

Isolated compounds exhibited substantial antibacterial activities (Table 1). Conyzolide (**1**) showed comparatively better and significant antibacterial activities against *E. coli* (MIC: 25 μg/mL). It also revealed considerable activities against *S. aureus* (MIC: 50 μg/mL) *P. aeruginosa* (MIC: 100 μg/mL) and *S typhi* (MIC: 100 μg/mL). However, Conyzoflavone (**2**) showed significant activity against S. *typhi* (MIC: 50μg/mL) in addition to its weak to moderate activity against all the tested pathogens.

Antifungal activity

Similarly, both compounds exhibited significant antifungal activities against the tested fungi (Table 1). Conyzoflavone (**2**) also found significantly active against *T. longifusus* (MIC: 10 μg/mL). Among the fungal strains, *T. longifusus* and *C. albicans* were most susceptible fungal pathogens. On the other hand, Conyzolide (compound **1**) showed comparatively weak antifungal activity as given in Table 1.

Table 1. Antimicrobial activity of the isolated compounds represented as Minimum Inhibitory Concentration (MIC, mg/mL).

	Minimum Inhibitory Concentration (MIC, μg/mL)		
Microorganism	Std. drug	Conyzolide (1)	Conyzoflavone (2)
Escherchia .coli	10[1]	25	50
Bacillus subtilis	8[1]	150	100
Shigella flexeneri	10[1]	125	100
Staphylococcus aureus	10[1]	50	100
Pseudomonas aeruginosa	9[1]	100	150
Salmonella typhi	15[1]	100	100
Trichophyton longifusus	1.4[2]	100	10
Candida albicans	1.8[2]	350	50
Aspergillus flavus	2.3[3]	300	200
Fusarium solani	1.1[2]	150	50
Candida glaberata	0.5[2]	400	100

[1]Std. drug: streptomycin, [2]Std. drug: = Miconazole, [3]Standard Drug = Amphotericin B.

Discussion

Fungal and bacterial infections are yet posing serious challenges to the human beings throughout the world[15–23]. Newly emerging resistance to the currently available drugs on the market is the drastic facet of this dilemma. Medicinal chemists and microbiologists are in search of new potent and effective antimicrobial agents to fight the pathogenic microorganisms. In the current study we have reported significant data highlighting the antimicrobial potential of *C. candensis* as a source of new lead compounds. The crude extract exhibited significant activity which was retained in the fractions with fluctuations. In case of antibacterial activity, the ethyl acetate fraction showed good activity against *E. coli* while the isolated compounds especially Conyzolide (**1**) revealed significant activity it. *E. coli* is one of the causative factors of urinary infections and most probably Conyzolide seems to be one of the compounds responsible for the folk use of *C. candensis* in urinary infections of the plant extract. Similarly both compounds showed considerable activity against *P. aeruginosa, S aureus* and S. *typhi.* Resistance to *S aureus* is one of the most serious problems in patients. Its prompt control needs careful selections of therapeutic options. The current data shows the potential of Conyzolide to further developed/modified as a lead compound against bacterial infections.

In case of antifungal activity extract, fractions and both the isolated compounds revealed significant results. *T longifusus*.is a common fungal pathogens of foot and skin infections. However *C. albicans* is responsible for majority of system and local infections and is one of the most hazardous fungal pathogens. The tested constituents of *C. candensis* were found substantially active against both the microorganisms. However their antifungal profile could be enhanced by developing new derivatives followed by 3-dimentional quantitative structure activity relationships (QSAR) analysis ultimately leading to an optimized compound. Both the compounds revealed preliminary safety profile against selected cell lines, which appears to be a positive indicator for thier further development/optmization. In short this study represents the pivotal importance of *C. candensis* to be a valuable and rich source of new antimicrobial agents against both the fungal and bacterial infections.

Declaration of interest

Authors have no commercial interests regarding publishing scientific data in this article.

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