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RESEARCH ARTICLE

Antimicrobial activity of confertifolin from *Polygonum hydropiper*

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Abstract
Confertifolin (6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c] furan-3 (1*H*)-one) was isolated from the essential oil of *Polygonum hydropiper* L. (Polygonaceae) leaves using column chromatography. Confertifolin showed activity both in bacteria and fungi. The lowest MIC for bacteria was observed against *Enterococcus faecalis* (31.25 µg/mL). Significant MIC for fungi was observed against *Scopulariopsis* sp (7.81 µg/mL), *Curvularia lunata* (7.81 µg/mL), *Epidermophyton floccosum* (7.81 µg/mL), *Trichophyton mentagrophytes* (16.62 µg/mL), *Trichophyton rubrum* (MTCC 296) (16.62 µg/mL), *Aspergillus niger* (31.25 µg/mL), *Botrytis cinerea* (31.25 µg/mL) *Magnaporthe grisea* (62.5 µg/mL), *Trichophyton simii* (125 µg/mL) and *Trichophyton rubrum* (clinical isolate) (125 µg/mL).

Keywords: *Antibacterial; antifungal; essential oil; confertifolin;* Polygonum hydropiper*; MIC*

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Introduction

Medicinal plants are used to treat various infectious diseases. Many plants used today were known to people of ancient cultures throughout the world and they were valued for their preservative and medicinal powers. Plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Reynolds, 1996; Lis-Balchin & Deans, 1997).

The search for biologically active extracts based on traditionally used plants is still relevant due to the development of microbial resistance to many antibiotics and the occurrence of fatal opportunistic infections. Essential oils with antimicrobial properties from medicinal as well as other edible plants have been recognized since antiquity (Harkenthal et al., 1999). The essential oils of many plant species and herbs have a broad spectrum of *in vitro* antimicrobial activities attributed to the high content of phenolic derivatives (Aziz et al., 1998).

Polygonum hydropiper L. (Polygonaceae) is an erect herb; it is used as a kind of spice by the Chinese, to flavor Southeast Asian-style foods such as laksa, and also by the Malays to flavor some of their traditional dishes. Moreover, it has long been used as a hot-tasting spice in Japan, China, and Europe, and also used as a folk medicine against cancer (Hartwell, 1970). Juice of the leaves is used to treat headaches, pain, toothache, liver enlargement, gastric ulcers, dysentery, loss of appetite, and dysmenorrhea; roots are used as a stimulant; juice is applied to wounds, skin diseases, and painful carbuncles (Ghani, 1998). The ethyl acetate fraction of this plant possesses antinociceptive activity (Rahman et al., 2002; Peng et al., 2003). The whole plant either on its own or mixed with other herbs, is used in the treatment of diarrhea, dyspepsia, itching skin, excessive menstrual bleeding, and hemorrhoids (Duke & Ayensu, 1985; Chevalier, 1996). Many drimane-type sesquiterpenes, such as polygodial and warburganal were isolated from *P. hydropiper* which possessed strong insect antifeedant and antibacterial activities (Barnes & Loder, 1962). Antioxidant flavonoids were also found from this plant (Zhao et al., 2003). Hydropiperoside a novel coumaryl glycoside, drimane-type sesqui norsesquiterpenoids, and polygonolide were reported

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(Furuta et al. 1986; Fukuyama et al., 1980, 1983, 1985). In this study, we examined the antimicrobial activity of confertifolin isolated from the essential oil of leaves of *P. hydropiper*.

Materials and methods

Plant material

The leaves of *Polygonum hydropiper* were collected from the Jarkand district of India in 2005. The plant was identified by Dr. D. Narasimmhan, Department of Botany, Madras Christian College, Chennai, India. The voucher specimen (ERI-75) was deposited at Entomology Research Institute, Loyola College, Chennai, India.

Isolation of essential oil

Fresh leaves were crushed and steam distilled using a clevenger-type apparatus with condenser. Distillation was continued for 3 to 5 h at 100°C, and the volatile compounds containing the water soluble fractions were allowed to settle for 30 min. The essential oil layer was separated and finally purified through micro filtering.

Gas chromatography-mass spectrometry (GC-MS)

The essential oil was quantified using gas chromatograph (GCMS-Shimadzu) equipped with a CPB-capillary column (mm inner diameter X 50 m length) mass spectrometer (ion source 200°C, RI 70 eV) programmed at 40°–280°C with a rate of 4°C/min. Injector temperature was 280°C; carrier gas was He (20 psi).

Isolation of the active compound

The essential oil of *P. hydropiper* was packed in a column chromatograph over silica gel acme's (100-200 mesh) in hexane. The column elution with hexane:ethyl acetate gradient solvent system with increasing polarity yielded 117 fractions (each 50 mL). Based on similar TLC patterns, fractions were combined and labeled; 16 fractions were finally obtained. Fraction 8 showed a single spot on TLC over the silica gel and crystallized using a hexane:ethyl acetate solvent system.

Identification of the active compound

The crystal was subjected to crystallographic analysis for structural determination. The X-ray data for the crystal were recorded using Bruker-AX, X-ray diffractometer at the Indian Institute of Technology, Chennai, India. A needle-shaped crystal was cut to a size of $0.3 \times 0.2 \times 0.4$ mm. It was tested for single crystalline using Leica-LSP polarising microscope. The single crystal was mounted on a BRUKER AXS kappa apex diffractometer. The structure was solved through the divert methods using the programme SIR 92 (WINGX) least squares refinement with anisotropic thermal parameter.

Antimicrobial activity

Microorganisms

Bacteria *Bacillus subtilis* MTCC 441, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* MTCC 3615, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 15380, *Protius vulgaris* MTCC 1771, *Pseudomonas aeruginosa* ATCC 27853 and *Erwinia* sp. MTCC 2760, and fungi *Trichophyton rubrum* MTCC 296, *Trichophyton rubrum* (clinical isolate), *Trichophyton mentagrophytes*, *Trichophyton simii*, *Epidermophyton floccosum*, *Scopulariopsis* sp. *Aspergillus niger* MTCC 1344, *Botrytis cinerea, Curvularia lunata* and *Candida albicans* MTCC 227 were used for the experiment. The above cultures were obtained from the Department of Microbiology, Christian Medical College (CMC), Vellore, Tamil Nadu, India.

Minimum inhibitory concentration (MIC)

The antimicrobial activity of confertifolin was assessed against bacteria and fungi using the broth microdilution method (Duraipandiyan & Ignacimuthu, 2007). The compound was dissolved in water + 2% dimethyl sulfoxide (DMSO). The initial concentration of the compound was 0.5 mg/mL. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 μL of suspension containing 10^8 CFU/mL of bacteria and 5 μL of 10^4 spore/mL of fungi, respectively. Fluconazole and Ketoconazole for fungi, and streptomycin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 24, 48, or 72 h at 27°C, while for bacteria the plates were incubated for 24 h at 37°C. MIC for fungi was defined as the lowest extract concentration showing no visible fungal growth after incubation time (NCCLS, 2002). The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. The experiment was repeated twice.

Results

GC-MS analysis of oil revealed the presence of six components. The components were: acetic acid (36.03%), propanoic acid, ethyl ester (18.21%), *n*-propyl

acetate (20.67%), ethylbenzene (0.93%), diethyleneglycol monoacetate (1.24%) and confertifolin (6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c] furan-3 (1*H*)-one) (22.91%), respectively (Table 1). This is the first report.

The crystal obtained through column chromatography was subjected to single XRD for structure determination. The XRD data confirmed the compound (Figure1) as confertifolin (6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c] furan-3 (1*H*)-one); the molecular formula is $C_{15}H_{22}O_2$; the melting point (mp) is 131°C; the molecular weight is 233.24. Previously, the same compound was reported from the bark of *Drimys winteri* Forst. (Winteraceae) (Appel et al., 1960).

Confertifolin was tested against the bacteria using the microdilution method. The lowest MIC was observed against *E. faecalis,* but higher concentration was needed against S. *epidermidis*, *P. aeruginosa, P. vulgaris B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *Erwinia* sp., and *S. typhi* (Table 2).

Table 1. The essential oil constituents of *Polygonum hydropiper* leaf.

Peak no.	Compound	Rt	(%)
1	Acetic acid	10.323	36.03
2	Propanoic acid, ethyl ester	13.882	18.21
3	n-Propyl acetate	14.474	20.67
4	Ethylbenzene	21.884	0.93
5	Diethyleneglycol monoacetate	28.676	1.24
6	Confertifolin(6,6,9a-trimethyl-4, 5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c] furan-3 (1*H*)-one)	31.468	22.91

Note: GC-MS analysis of essential oil indicating the compounds.

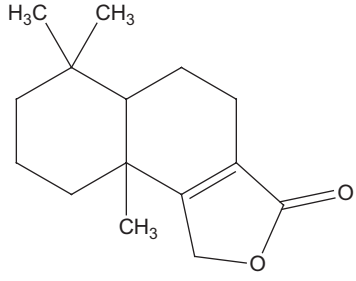

Figure 1. Confertifolin (6,6,9a-trimethyl-4, 5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c] furan-3 (1*H*)-one) isolated from *Polygonum hydropiper*.

Confertifolin was tested against pathogenic fungi. We observed significant antifungal activity (Table 3). The MIC values varied depending on the least resistant to most resistant species: *Scopulariopsis* sp., *C. lunata*, *E. floccosum*, *T. mentagrophytes*, *T. rubrum* MTCC 296, *A. niger, B. cinerea*, *M. grisea*, *T. rubrum* (clinical isolate) and *T. simii*.

Discussion

Confertifolin showed a significant MIC against *E. floccosum* (7.81 µg/mL). Antifungal activity of polygodial isolated from *P. hydropiper* showed MIC against *T. rubrum*, *E. floccosum* but did not inhibit the growth of *T. mentagrophytes*, *Microsporum canis,* and *Microsporum gypseum* up to 100 µg/mL (Malheiros et al., 2005). Compared to this, confertifolin showed lowest MIC against *T. rubrum* (16.62µg/mL). *Polygonum punctatum* was found to be active against *Candida albicans* and dermatophytes (Alves et al., 2001). Hammer et al. (1999) investigated 52 plant oils and extracts against *Acinetobacter baumannii*, *Aeromonas veronii*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Staphylococcus aureus*. The essential oil isolated from *Syzygium cumini* and *Syzygium travancorium* leaf was tested against bacteria (Shafi et al., 2002). Confertifolin possessed significant antifungal activity against dermatophytes. Confertifolin, isolated from the essential oil of the leaf of *Polygonum hydropiper,* showed moderate antibacterial activity. However, it showed significant antifungal

Table 2. Antibacterial activity of confertifolin isolated from *Polygonum hydropiper.*

Tested compounds	Minimum inhibitory concentration (MIC) µg/mL								
	B.s[c]	*S.a*	*S.e*	*E.f*	*E.c*	*P.a*	*K.p*	*Er*	*P.v*
Confertifolin[a]	>500	>500	500	31.25	>500	500	>500	>500	500
Streptomycin[b]	25	6.25	>50	25	12.5	25	6.25	1.56	nt

[a]Isolated compound; [b]Standard; [c]Bacteria: *B.s, Bacillus subtilis; S.a, Staphylococcus aureus; S.e, Staphylococcus epidermidis; E.f, Enterococcus faecalis; E.c, Escherichia coli; P.a, Pseudomonas aeruginosa; K.p, Klebsiella pneumoniae; Er, Erwinia sp; P.v, Proteus vulgaris*; nt: not test.

Table 3. Antifungal activity of confertifolin isolated from *Polygonum hydropiper*.

Tested compounds	Minimum inhibitory concentration (MIC) µg/mL										
	T.m[e]	*E.f*	*T.s*	*C.l*	*A.n*	*B.c*	*T.r296*	*M.g*	*T.r (cl)*	*Scr*	*C.a*
Confertifolin[a]	16.62	7.81	125	7.81	31.25	31.25	16.62	62.5	125	7.81	>250
Fluconazole[d]	25	12.5	<12.5	<12.5	100	nt	<12.5	nt	25	<12.5	>100
Ketoconazole[d]	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	>100

[d]Standard antifungal agent; [e]Fungi: *T.m, Trichophyton mentagrophytes*; *E.f, Epidermophyton floccosum*; *T.s, Trichophyton simii*; *C.l, Curvularia lunata*; *A.n, Aspergillus niger*; *B.c, Botrytis cinerea*; *T.r, Trichophyton rubrum* (MTCC 296); *M.g, Magnaporthe grisea*; *T.r, Trichophyton rubrum* (clinical isolate); *Scr, Scopulariopsis* sp.; *C.a, Candida albicans;* nt, not tested.

activity. This is the first report on the antifungal activity of confertifolin.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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