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Research Article

ECBOLIN A: A BIOACTIVE COMPOUND FROM THE ROOTS OF *ECBOLIUM VIRIDE* (FORSSK.) ALSTON

K FRANCINA CECILIA [1], R RAVINDHRAN [1*], V DURAIPANDIYAN [2]

[1] Department of Plant Biology and Biotechnology, Loyola College, Chennai 600 034, India,[2] Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
Email: raviloyola1998@gmail.com

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ABSTRACT

The active compound Ecbolin A (a furofuran type of lignan) was isolated from ethyl acetate extract of *E. viride* roots using chromatographic techniques. Minimum Inhibitory Concentration (MIC) of the active compound was studied against nineteen bacteria and twelve fungi using micro-broth dilution technique. The results showed significant antibacterial and moderate antifungal activity. Thus the present study reveals the use of Ecbolin A as a new effective antibiotic against multidrug resistant strains.

Keywords: Green Shrimp; Antimicrobial; Ecbolin A; MIC

INTRODUCTION

Plants have been a valuable source of natural products for maintaining human health for a long period of time. The search for compounds with antimicrobial activity from plants has gained importance due to growing worldwide concern about the increase in the rate of infection by antibiotic resistant microbes[1]. Therefore, the study on unexplored medicinal plants will lead to the development of more potent drugs with no or minimal toxicity and high sensitivity towards the emerging microbial agents[2,3].

Ecbolium viride (Forssk.) Alston (Acanthaceae) is a perennial woody undershrub (also known as Green Shrimp) found in the plains of India and also in Arabia, Malaysia, Sri Lanka and Tropical Africa. Leaves, flowers and roots contain flavones, glycoflavones, luteolin, orientin, isoorientin and vitexin[4]. This plant is widely used in Indian traditional medicinal system (Siddha, Ayurveda, Unani and Folk)[5,6]. In folk medicine, aqueous extract of dried roots of the plant are used for menorrhagia[7,8]. The tribes (Tripura and Paliyar) use roots for the treatment of jaundice[9] and rheumatism[10] while the roots and leaves together are used against tumour [11].

Roots of *E. viride* possess antioxidant[12] , anti-inflammatory[13], anti-hepatotoxicity [14,15], antiplasmodial, antitrypanosomal and antimalarial activity[16]. Previous studies have shown the presence of alkaloids, carbohydrates, glycosides, tannins and saponins[17]. Thus phytochemical and pharmacological studies on *E. viride* support its traditional uses. However, no reports are available on the antimicrobial activity of compound Ecbolin A isolated from the roots of *E. viride.* Hence the present study was carried out to study the antimicrobial potential of Ecbolin A (lignan) isolated from the ethyl acetate extract of *E. viride* roots.

MATERIALS AND METHODS

Plant Material

Roots of *E. viride* were collected during summer (June) of 2011 from Srirangam, Trichy, Tamil Nadu, India. It was authenticated by Dr. P. Jayaraman, Scientist from the Plant Anatomy Research Centre (PARC) Tambaram, Tamil Nadu, India. A voucher specimen of the plant (PARC/2012/1152) was deposited in the herbarium of Department of Plant Biology and Biotechnology, Loyola College, Chennai, India.

Extraction and Isolation

The roots were cleaned, shade dried at room temperature and then milled to a fine powder (2 kg) in manual mill and stored in closed containers in the dark until further use. The extraction was carried out with different solvents in the increasing order of polarity, namely: n-hexane, ethyl acetate and methanol by soaking the material in respective solvents (1:3 w/v) for 48 h at room temperature. The extract was filtered through Buchner funnel with Whatman number 1 filter paper and the filtrate was condensed in the rotary evaporator (Equitron, India). The extraction with different solvents was carried out sequentially. The yield of extracts was n-hexane (23 g), ethyl acetate (40 g) and methanol (45 g). The crude ethyl acetate extract of roots (40 g) was subjected to column chromatography over silica gel (200 g- Qualigens 100 - 200 mesh) and eluted with n-hexane followed by combinations of n-hexane : ethyl acetate (95:5 to 0:100) and ethyl acetate : methanol (95:5 to 0:100). The fractions were eluted with solvents ranging from non polar to polar at the rate of 30 drops per minute in a 100 ml conical flask. Elution of the column with n-hexane : ethyl acetate (8:2) (Fraction 7) showed a crystal (2.5 g). It gave a single spot on TLC over silica gel (Rf - 0.3) with n-hexane: ethyl acetate (8:2) as the developing system. Physical and spectroscopic data (UV, FT-IR, ^{1}H NMR, ^{13}C NMR and ESI-MS) were compared with those reported in literature [18].

Microbial Organisms

The following bacteria and fungi were used for the experiment. Gram negative bacteria: *Escherichia coli* (ATCC 25922), *Erwinia amylovora* (MTCC 2760), *Klebsiella pneumoniae* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (MTCC 1771), *Proteus mirabilis* (ATCC 49565), *Salmonella paratyphi-B* and *Vibrio cholerae* (ATCC 14035), *Shigella flexneri* (MTCC 1457). Gram positive: *Bacillus subtilis* (ATCC 441), *Enterococcus faecalis* (ATCC 29212), *Enterobacter aerogens* (MTCC 111), *Micrococcus luteus* (ATCC 4698), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615). Clinical isolates: *Escherichia coli* (ESBL-3904), *Klebsiella pneumoniae* (ESBL - 3971), *Staphylococcus aureus* (MRSA - methicillin resistant) and *Enterococcus durans* (P502). Fungal strains: *Candida albicans* (MTCC 227), *Candida krusei, Candida tropicalis*, *Microsporum gypseum, Malassezia pachydermatis*, *Trichophyton rubrum* 57/01, *Trichophyton mentagrophytes* 66/01, *Epidermophyton flocussum* 73/01, *Scopulariopsis* sp. 101/01, *Aspergillus flavus*, *Botyritis cinerea, Curvularia lanata* 46/0. All the microbial cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

Preparation of inoculum

Bacterial inoculum was prepared by growing the cells in Mueller Hinton Broth (MHB) (Himedia, India) for 24 h at 37 °C. The cell suspension was diluted with sterile MHB to provide initial cell count of 10^{8} CFU/ml. The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28 °C for 10 days and the spores were collected using sterile double distilled water and homogenized.

Determination of antimicrobial activity of Ecbolin A

The minimum inhibitory concentration (MIC) test was performed according to the standard reference methods for bacteria[19],

filamentous fungi[20] and yeasts[21]. The compound was dissolved in water + 2 % dimethyl sulfoxide (DMSO). The initial concentration for the compound was 250 µg /ml and it was serially diluted two fold. Each well was inoculated with 100 µl of suspension containing 10^8 CFU/ml of bacteria and 10^4 spores/ml of fungi, respectively. The antibacterial agent ciprofloxacin and antifungal agent fluconazole were included in the assay as positive controls. For fungi, the plates were incubated for 24, 48 or 72 h at 28 °C upto 9 days for dermatophytes while bacterial plates were incubated for 24 h at 37 °C. MIC was defined as the lowest extract concentration showing no visible fungal growth after incubation time. Tested broth of 5 µl was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. All the experiments were performed in triplicates and the results were averaged.

RESULTS AND DISCUSSION

In the present study, ethyl acetate extract exhibited significant activity against bacteria and fungi when compared to other solvent extracts. Hence it was subjected to column chromatography and 16 fractions were obtained. Fraction 7 yielded a single crystal and was identified as Ecbolin A, a furofuran type of lignan (Fig. 1). The electron impact mass spectrum of Ecbolin A indicated the molecular weight of 444.05 corresponding to the molecular formula $C_{23}H_{24}O_9$. The structure of Ecbolin A reported in our study was compared and confirmed with previous reports[18]. The compound Ecbolin A inhibited the growth of gram positive bacteria such as *S. aureus* (7.812 µg/ml), *B. subtilis* (62.5 µg/ml), gram-negative bacteria *K. pneumoniae* (62.5 µg/ml), *E. amylovora* (15.625 µg/ml), *V. cholerae* (31.25 µg/ml) and a clinical isolate *S. aureus* (31.25 µg/ml) (Table 1). The compound also inhibited the growth of fungi such as *M. pachydermatis* (62.5 µg/ml), *C. albicans* (125 µg/ml) and *Scopulariopsis* sp. (250 µg/ml) (Table 2). Previous reports have described the effective antimicrobial activity displayed by several lignans. A lignan, (+)-lyoniresinol-3a-O-b-D-glucopyranoside isolated from the root bark of *Lycium chinense*, exhibited potent antimicrobial activity against *S. aureus* and three human-pathogenic fungi, *C. albicans, S. cerevisiae* and *T. beigelii*[22]. Another lignan, hopeanolin from the stem bark of *Hopea exalata* showed high antifungal activity against *A. solani, C. lagenarium, F. oxysporum, P. oryzae* and *V. mali*[23]. The results of the present study are consistent with the earlier reports involving lignans as a natural compound for the development of antibiotics.

Table 1: Antibacterial activity (MIC) of Ecbolin A isolated from *Ecbolium viride*

Tested organisms	Ecbolin A (µg/ml)	Ciprofloxacin (µg/ml)
Gram positive		
Bacillus subtilis	62.5	<0.78
Enterococcus faecalis	>250	6.25
Enterobacter aerogens	>250	<0.78
Staphylococcus aureus	7.812	<0.78
Staphylococcus epidermidis	250	6.25
Micrococcus luteus	>250	>100
Gram negative		
Escherichia coli	>250	>100
Erwinia amylovora	15.625	<0.78
Klebsiella pneumoniae	62.5	<0.78
Pseudomonas aeruginosa	>250	100
Proteus vulgaris	250	nt
Proteus mirabilis	250	nt
Vibrio cholerae	31.25	<0.78
Salmonella paratyphi-B	>250	6.25
Shigella flexneri	250	<0.78
Clinical isolates		
Escherichia coli	>250	<0.78
Klebsiella pneumoniae	250	<0.78
Staphylococcus aureus	31.25	<0.78
Enterococcus durans	>250	nt

Values are the mean of three repeat experiments; nt: not test; Ciprofloxacin (standard antibacterial agent); Ecbolin A (isolated compound).

Table 2: Antifungal activity (MIC) of Ecbolin A isolated from *Ecbolium viride*

Tested organisms	Ecbolin A(µg/ml)	Fluconazole (µg/ml)
Dermatophytes		
Candida albicans	125	>100
Candida krusei	>250	>100
Candida tropicalis	>250	>100
Microsporum gypseum	>250	nt
Malassezia pachydermatis	62.5	12.5
Trichophyton rubrum	>250	25
Trichophyton mentagrophytes	>250	25
Epidermophyton flocussum	>250	12.5
Scopulariopsis sp.	250	<12.5
Aspergillus flavus	>250	25
Opportunistic pathogens		
Botyritis cinerea	> 250	nt
Curvularia lanata	> 250	<12.5

Values are the mean of three repeat experiments; nt: not test; Fluconazole (standard antifungal agent); Ecbolin A (isolated compound).

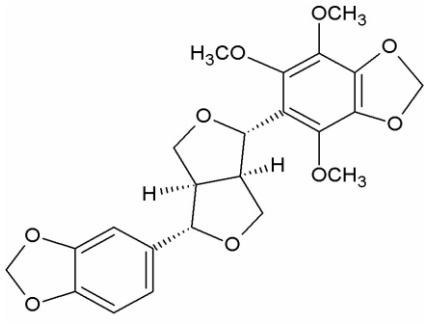

Fig.1: Ecbolin A - 6-(3,4-methylenedioxyphenyl)-2-(2,5,6-trimethoxy-3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0] octane isolated from *Ecbolium viride* roots

Thus the present study showed that the isolated lignan, Ecbolin A exhibited promising antimicrobial activity. This plant could be useful for the development of new antimicrobial drug. This is the first report on the antimicrobial activity for Ecbolin A isolated from *E. viride*.

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