Journal of Enzyme Inhibition and Medicinal Chemistry, December 2006; 21(6): 765–769

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healthcare

Antimicrobial bioassay of *colchicum luteum* baker

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(Received 20 February 2006; accepted 13 June 2006)

Abstract
The methanolic extract of the corms of *Colchicum luteum* Baker (Liliaceae) and its subsequent fractions in different solvent systems were screened for antibacterial and antifungal activities. The crude extract and all the fractions demonstrated moderate to excellent antifungal activities against tested pathogens in antifungal bioassay. Excellent antifungal activity was shown against *trichophyton longifusus*, up to 75%, and *microsporum canis*, up to 85%, while the crude extract and subsequent fractions showed mild to moderate activities in an antibacterial bioassay with maximum antibacterial activity 58% against *Bacillus subtilis*.

Keywords: Colchicum luteum *Baker, antibacterial, antifungal*

Introduction

Many efforts have been made to discover new antimicrobial compounds from a variety of sources such as microorganisms, animals and plants. Scientific experiments on the antimicrobial properties of plants and their components have been documented since the late 19th century [1]. Over the last decade, biological control has gained tremendous importance over chemical antimicrobials [2]. Even commonly used foodstuffs contains a number of naturally occurring antimicrobials that help in the prevention of their deterioration [3].

The increasing prevalence of multidrug-resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies [4]. Plant based antimicrobials have a considerable safety profile and are not associated with many side effects when compared with synthetic products. Additionally, they have an enormous therapeutic potential to heal many infectious diseases [5].

Pakistan occupies a unique position among developing countries as it has a variety of medicinal plants due to its varied climate, and is rich in valuable medicinal plant [6]. The current need is to convert this valuable heritage into practice, as very little work has been done on Pakistani medicinal plants [7] to meet the growing demand of the local market as well as to earn foreign exchange from their export.

The plant *Colchicum luteum* Baker, commonly known as Suranjan-e-Talkh (Urdu) and Meadow saffron (English), belongs to the Liliaceae family [8]. The Liliaceae are mostly perennial herbs with starchy rhizomes, corms, or bulbs comprising about 280 genera and 4,000 species. The plant usually requires a very sunny position and plants can take 4 – 5 years to flower when grown from seed [9]. The genus colchicum includes 42 species, most of which are endemic to the Middle East [10], and South Africa to Western Europe and Asia [11]. The corms of *Colchicum luteum* Baker are extensively used for the treatment of gout, rheumatism and diseases of the liver and spleen [12] also have alterative, aphrodisiac, carminative and laxative properties; they are also used as a blood purifier. Excellent enzyme inhibition activity is also shown by the crude methanolic extract and subsequent fractions of *Colchicum luteum* against lipoxygenase [13,26]. Phytochemically, the mainstay of the genus colchicum is alkaloids including *Colchicum luteum* [14] and from colchicum, thirty-one different alkaloids are been isolated [15].

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ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360600900547

Colchicine is the main alkaloid isolated from all species of the genus, colchicum [16].

Methods and materials

Plant material

Colchicum luteum Baker, as a whole plant was collected from Sherengel, Upper Dir, N.W.F.P (Pakistan) during the month of Feb-March 2004. The plant material was identified by Professor Dr Jahandar Shah, plant taxonomist and Vice Chancellor Malakand University, and verified by Professor Dr Abdur-Rashid, Department of Botany, University of Peshawar.

Extraction

The shade-dried plant material was chopped into small pieces and pulverized into a fine powder. The powdered plant material (10 Kg) was soaked in methanol, with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The process was repeated thrice and the filtrate was concentrated under vacuum at low temperature (40°C) using a rotary evaporator to give a yellowish crude extract (246 g).

Fractionation

The crude methanolic extract (246 g) was suspended in distilled water (500 mL) and partitioned with *n*-hexane (3 × 500 mL), chloroform (3 × 500 mL), ethyl acetate (3 × 500 mL) and *n*-butanol (3 × 500 mL) to yield the *n*- hexane (26 g), chloroform (59 g), ethyl acetate (32 g), *n*-butanol (35 g) and aqueous (68 g) fractions, respectively.

Antifungal bioassay

Antifungal activity of the crude extract and various fractions were evaluated by the agar tube dilution method [17]. The samples at a concentration of 24-mg/ mL were dissolved in sterile (autoclaved) dimethyl sulfoxide (DMSO, Merck), which served as a stock solution. Sabouraud dextrose agar (SDA, Sigma-Aldrich, Germany) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4.0 g of agar-agar in 500 mL distilled water and stirring with a magnetic stirrer to dissolve it. Then 4 mL amounts were dispensed into screw cap tubes, which were autoclaved at 120°c for 15 min and then cooled to 15°C. The non-solidified SDA media was mixed with the stock solution (66.6 µl) giving a final concentration of 400 µg of the extract per ml of SDA. Tubes were then allowed to solidify in the slanted position at room temperature. Each tube was inoculated with a piece (4 mm diameter) of inoculums removed from a seven days old culture of fungi for non-mycelial growth; an agar surface streak was employed. Other media supplemented with dimethyl sulfoxide (DMSO) and reference antifungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed after 7-days of incubation at 28 ± 1°C. Humidity (40–50%) was controlled by placing an open pan of water in the incubator. After incubation for 7-days, the test tubes were analyzed for visible growth of the microorganisms.

Antibacterial bioassay

The crude extract and its various fractions were also screened against various human pathogens including *Escherichia coli, Bacillus subtilis, Klebsiella pneumonae, Shigella flexenari, Staphylococcus aureous, and Salmonella typhi* by the agar well diffusion method [17]. In this method, 10 mL aliquots of nutrients broth (Sigma-Aldrich, Germany) were inoculated with the test organism and incubated at 37°C for 24 h. Using a sterile pipette, 0.6 mL of the broth culture of the test organism was added to 60 mL of molten agar, which had been cooled to 45°C, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 ml of the culture was added to 20 ml of agar). Duplicate plates of each organism were prepared. The agar was allowed to set and harden and the required number of wells were dug in the medium with the help of a sterile metallic cork borer ensuring proper distribution of the wells in the periphery and one in the center. Agar plugs were removed. Stock solutions of the test samples at a concentration of 1 mg/ml were prepared in sterile dimethyl sulfoxide (DMSO) and 100 µl and 200 µl of each dilution was added in their respective well. Control well received only 100 µl and 200 µl of DMSO. Imipinem was used as a standard drug. The plates were left at room temperature for 2 h to allow diffusion of the samples then incubated face upwards at 37°C for 24 h. The diameter of the zones of inhibition was measured to the nearest mm (the well size also being noted).

Results and discusion

WHO estimates that approximately 80% of the developing world's populations meet their primary health care needs through traditional medicine [18], despite the fact, that there is the possibility that the herb used is harmful to the patient, if wrong plant parts or wrong concentrations are used [19]. Therefore, the global interest in natural products remedies is their standardization. For this purpose, the crude methanolic extract and subsequent fractions of *Colchicum luteum* Baker were screened for antibacterial and antifungal activities.

Antifungal activities of the crude extract and subsequent fractions of the *Colchicum luteum* were evaluated for *Trichophyton longi fusus*, *Candida albicans*,

Aspergilus flavus, *Microsporum canis*, *Fusarium solani*, and *Candida glaberata*, in comparison with miconazole and amphotericin-B. Growth in the medium containing the extract was determined by measuring the linear growth in mm and % growth was calculated with reference to the negative control. The antifungal results are displayed in (Table I) and the crude extract and its various fractions showed good to excellent activity against *Trichophyton longi fusus*. The *Trichophyton longi fusus* belongs to the genus Trichophyton. Trichophyton is a dermatophyte, which is the causative agent of dermatophytosis and infects the hair, skin, and nails [20,22] and *Trichophyton* species may cause invasive infections in immunocompromised hosts [23]. The crude extract showed (56%) inhibition, while the chloroform fraction displayed significant antifungal activity (70%). Interestingly, both the ethyl acetate and n-butanol fractions each exhibited excellent results, (75%). However, only (35%) antifungal activity is shown by the aqueous fraction. Overall poor activity against *Candida albicans* was shown except for the *n*-butanol fraction (60%). The crude extract and chloroform fraction showed no activity, while the ethyl acetate and aqueous fractions gave 35% and 25% activity respectively. In case of *Aspergilus flavus*, the crude extract and aqueous fraction showed no activity, the chloroform and *n*-butanol fractions showed (65%) and (50%) respectively, while the ethyl acetate fraction showed excellent (70%) activity. Only the aqueous fraction showed low (25%) antifungal activity against *Microsporum canis*, which causes the infections of hair, skin and nail and immunocompromised patients are also infected [24,24,25], whereas the crude extract displayed good (65%) activity, while the chloroform fraction demonstrated significant (70%) activity and both the ethyl acetate and *n*-butanol fractions displayed excellent (85%) and (75%) activity respectively. No activity was shown by the chloroform, ethyl acetate and aqueous fractions against *Fusarium solani* while moderate activity is shown by the crude extract (50%) and *n*-butanol (55%) fraction. In case of *Candida glaberata,* no activity was displayed by the crude extract and chloroform fractions, low activity by the ethyl acetate fraction (35%) and poor (15%) activity by the aqueous fraction, but good activity was displayed by the *n*-butanol (60%) fraction

The crude extract and subsequent fractions of *Colchicum luteum* Baker were screened against various human pathogens including *E. coli, B. subtilis, K. pneumonae, S. flexenari, S. aurous, P. aeruginosa*, and *S. typhi* (Table II). The crude extract and various fractions of *Colchicum luteum* Baker showed low to moderate activity against *E.coli*; ethyl acetate fraction (57%), chloroform fraction (50%), crude extract displayed (43%) and *n*-butanol fraction (40%), while the aqueous fraction was devoid of any activity. In case of *B. subtilis*, the crude extract and various fractions

Table I. Anti-Fungal activities of crude extract & various fractions of *colchicum luteum* baker.

Name of Fungi	-ve control Linear Growth (mm)	Crude Extract		$CHCl_3$ Fraction		EtOAc Fraction		*n*-BuOH Fraction		Aqueous Fraction		Standard Drugs	
		Linear Growth (mm)	Inhibition %	Linear Growth (mm)	Inhibition %	Liner Growth (mm)	Inhibition %	Liner Growth (mm)	Inhibition %	Liner Growth (mm)	Inhibition %	Name	MIC (µg/ml)
Trichophyton longi fusus	100	44	56%	30	70%	25	75%	25	75%	65	35%	Miconazole	65
Candida albicans	100	100	0%	100	0%	65	35%	40	60%	75	25%	Miconazole	110.8
Aspergilus flavus	100	100	0%	35	65%	30	70%	50	50%	100	0%	Amphotericin-B	24
Microsporum canis	100	35	65%	30	70%	25	85%	25	75%	100	25%	Miconazole	97
Fusarium solani	100	50	50%	100	0%	100	0%	45	55%	100	0%	Miconazole	85
Candida glaberata	100	100	0%	100	0%	65	35%	40	60%	85	15%	Miconazole	110.8

Table II. Antibacterial activities of crude extract & various fractions of *colchicum luteum* baker.

Name of Bacteria	Zone of Inhibition of standard (Imipinem)	Crude Extract		$CHCl_3$ Fraction		EtOAc Fraction		*n*-BuOH Fraction		Aqueous Fraction	
		Zone of Inhibition (mm)	Inhibition %	Zone of Inhibition (mm)	Inhibition %	Zone of Inhibition (mm)	Inhibition %	Zone of Inhibition (mm)	Inhibition %	Zone of Inhibition (mm)	Inhibition %
Escherichia coli,	30	13	43%	15	50%	17	57%	12	40%	Nil	Nil
Bacillus subtilis	33	19	58%	18	55%	Nil	Nil	15	45%	16	48%
Klebsiella pneumonae,	27	14	52%	15	56%	11	41%	10	37%	Nil	Nil
Staphylococcus aureous	33	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Shigella flexenari	24	10	42%	08	33%	10	42%	12	50%	13	54%
Salmonella typhi	25	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

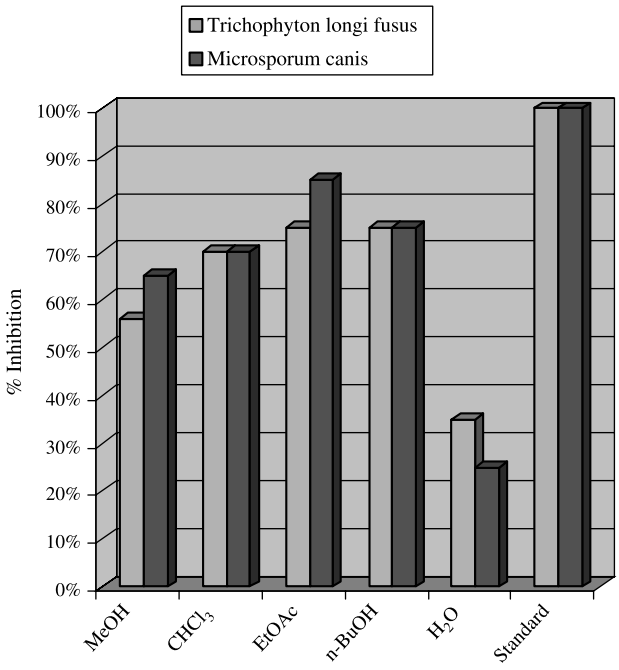

Figure 1. Antifungal activities of the crude extract and subsequent fractions of Colchicum luteum Baker.

showed low to moderate activity; crude extract (58%), chloroform fraction (55%), *n*-butanol fraction (45%), and aqueous fraction (48%) activity. while the ethyl acetate fraction was inactive. The antibacterial activity of the plant is low to moderate for *Klebsiella pneumoniae*; crude extract (52%), chloroform fraction (56%), ethyl acetate fraction (43%) and n-butanol fraction (37%) activity while no zone of inhibition was shown by the aqueous fraction. The antibacterial activity of the plant is low to moderate against *Shigella flexenari*; aqueous fraction (54%), n-butanol fraction (50%), crude extract (42%), chloroform fraction (33%) and the ethyl acetate fraction (42%) activity. The crude extract and various fractions displayed no antibacterial activity against *S. aurous* and *S. typhi*.

From the results shown in (Figure 1), it can be assessed that this plant species has excellent antifungal activity against *T. longifusus* and *M. canis*. Therefore, it could be a natural antifungal against these pathogens in different infections but there is a need to examine the safety profile of the plant.

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