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To cite this article: Haroon Khan, Murad Ali Khan, Tahira Mahmood & Muhammad Iqbal Choudhary (2008) Antimicrobial activities of *Gloriosa superba* Linn (Colchicaceae) extracts, Journal of Enzyme Inhibition and Medicinal Chemistry, 23:6, 855-859, DOI: [10.1080/14756360701747409](https://doi.org/10.1080/14756360701747409)

To link to this article: <https://doi.org/10.1080/14756360701747409>



Published online: 20 Oct 2008.



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Antimicrobial activities of *Gloriosa superba* Linn (Colchicaceae) extracts

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(Received 7 September 2007; accepted 26 September 2007)

Abstract

The methanol extract of the rhizomes of *Gloriosa superba* Linn (Colchicaceae) and its subsequent fractions in different solvent systems were screened for antibacterial and antifungal activities. Excellent antifungal sensitivity was expressed by the *n*-butanol fraction against *Candida albicans* and *Candida glabrata* (up to 90%) and against *Trichophyton longifusus* (78%) followed by the chloroform fraction against *Microsporum canis* (80%). In the antibacterial bioassay, the crude extract and subsequent fractions showed mild to moderate antibacterial activities. Chloroform fraction displayed highest antibacterial sensitivity against *Staphylococcus aureus* (88%) followed by the crude extract (59%). The total phenol content of the crude extract and fractions of the plant expressed no significant correlation with the antimicrobial activities.

Keywords: *Gloriosa superba* Linn, antibacterial, antifungal, total phenol content, rhizomes, extracts

Introduction

Considerable work have been done on various sources such as microorganisms, animals, and plants to design and develop new antimicrobial compounds for the treatment of microbial infections, both topical and systemic applications, as possible alternatives to chemically synthetic drugs [1]. Literature reports and ethnobotanical records suggest that plants are the sleeping giants of the pharmaceutical industry [2]. They may provide natural source of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally. But scientific studies on the antimicrobial properties of plants and their components have been documented in the late 19th century [3]. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts.

Finding healing powers in plants is an ancient idea and despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of

prescribed medicines in industrialized countries derived directly or indirectly from plants. However, plants used in traditional medicine are still understudied, particularly in clinical microbiology [4]. Recently, the biological control has gained tremendous importance over chemicals antimicrobials [5], mostly because of the side effects of the synthetic products. Even commonly used food stuff contains a number of naturally occurring antimicrobial agents that help in the prevention of the food material from deterioration [6]. 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 [7]. Plant-based antimicrobials have a considerable safety profile and are not associated with many side effects when compare with synthetic products. Additionally, they have an enormous therapeutic potential to heal many infectious diseases [8].

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The plant *Gloriosa superba* Linn is commonly known as climbing lily (English) and Samp ki Bothi (Urdu) and belongs to the *Colchicaceae* family. It is a perennial herb, semi-woody herbaceous, branching climber, reaching approximately 5 metres in height. 1 to 4 stems arise from single V-shaped fleshy cylindrical tuber/rhizomes. The perianth segments, which are accrescent during anthesis and become reflexed, are striking in color, yellow proximally and at margins and dark red in the median portion [9,10]. A native of tropical Africa and is now found growing naturally throughout much of tropical Asia including: India, Sri Lanka, Malaysia and Burma [11].

In traditional medicine practiced, it's important medicinal used is as a source of colchicine, and both seed and rhizome are used for the treatment of gout [12,13]. The juice of the leaves is used to kill head lice and also as an ingredient in arrow poisons, as well as antimosquito properties [14]. The rhizomes also have antidotal properties to snakebite and in India it is commonly placed on windowsills to deter snakes. Many cultures believe the species to have various magical properties [15–17]. The crude extract and fractions expressed outstanding inhibition on lipoxygenase. Phytochemically, in addition to colchicine and gloriosine, *Gloriosa superba* also contains other alkaloid such as 3-desmethyl colchicine, β -lumicolchicine, N-Formyl-desacetyl-colchicine, 2-desmethyl colchicine, and compounds like, chelidonic acid and salicylic acid [18].

Materials and methods

Plant material

The *Gloriosa superba* Linn, as a whole plant was collected from Chatter Bagh, Islamabad (Pakistan) during the month of June 2007. Authentication of the plant material was done by the Botany department of the PCSIR Laborites Peshawar and a voucher specimen was deposited there.

Extraction

The shade dried plant material (rhizomes) was chopped into small pieces and finally pulverized into fine powder. The powdered plant material (9 Kg) was soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator. A crude extract (269 g) was obtained.

Fractionation

The crude methanol extract (967g) was suspended in distilled water (500 mL) and sequentially 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 (123 g), chloroform (231 g), ethyl acetate (180g), *n*-butanol (203 g) and aqueous (161 g) fractions, respectively.

Antifungal bioassay

Antifungal activity of the crude extract and various fractions were evaluated by agar tube dilution method [19]. The samples at concentrations of 24mg/mL were dissolved in the sterile (autoclaved) dimethyl sulfoxide (DMSO, Merck), which served as 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. It was then stirred with a magnetic stirrer to dissolve it. Then 4mL amount was 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 poisoned with stock solution (66.6 μ L) giving the final concentration of 400 μ g 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 anti-fungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed after 7-days of incubation at $28 \pm 1^\circ\text{C}$. Humidity (40–50%) was controlled by placing an open pan of water in the incubator. After the incubation period for 7-days, the test tubes were analyzed for the visible growth of the microorganisms.

Antibacterial bioassay

The crude extract and its various fractions in concentration of 3mg/mL were also screened against various human pathogens including *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Staphylococcus aureus*, and *Salmonella typhi* by agar well diffusion method [19]. In this method, 10 mL aliquots of nutrients broth (Sigma-Aldrich, Germany) was 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 using 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

Table I. Anti-fungal activities of crude extract and various fractions of *Gloriosa superba* Linn.

Name of Fungi	N.C	Crude extract		CHCl ₃		Ethyl acetate		Butanol		H ₂ O		Std
		LG	%	LG	%	LG	%	LG	%	LG	%	
<i>T. longifusus</i>	100	48	52	33	67	33	67	22	78	82	18	65 ¹
<i>C. albicans</i>	100	35	65	25	75	20	80	10	90	100	–	110.8 ¹
<i>A. flavus</i>	100	–	–	–	–	–	–	–	–	–	–	24 ²
<i>M. canis</i>	100	30	70	20	80	30	70	25	75	100	–	97 ¹
<i>F. solani</i>	100	–	–	–	–	–	–	–	–	–	–	85 ¹
<i>C. glabrata</i>	100	40	60	20	80	10	90	10	90	70	30	110.8 ²

¹Standard Drug = Miconazole; ²Standard Drug = Amphotericin B, NC = Negative control, LG = Linear growth (mm), % = % inhibition.

the test samples at a concentration of 1mg/mL were prepared in the sterile dimethyl sulfoxide (DMSO) and 100µL and 200 µL of each dilution was added in their respective wells. Control well received only 100 µL and 200µL of DMSO. Imipinem was used as standard drug (10µg / mL). 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).

Total phenol content determination

According the standard method of Folin and Denis [20,21], the total phenol concentration of the crude extract and subsequent fractions of the *Gloriosa superba* Linn were determined. Briefly, each extract (10mg) was mixed with Folin-Denis reagent (5mL), Na₂CO₃ (20%, 10mL) and diluted by a factor 100 with distilled water. The resulting mixture was left at room temperature for 10 min after filtration and the absorbance was measured at 770 nm against the blank using Spectronic 20D (Milton Roy). The total phenol content of each extract and fraction was estimated by comparison with a standard curve generated using tannic acid.

Results and discussion

Traditional health care systems using medicinal plants can be recognized and used as a starting point for the development of novelties in drugs. For this purpose, crude methanol extract and subsequent fractions of *Gloriosa superba* Linn were screened for biological activities especially antibacterial and antifungal activities.

Anti-fungal activities of the crude extract and subsequent fractions of the *Gloriosa superba* Linn were evaluated for *T. longifusus*, *C. albicans*, *Aspergillus flavus*, *Microsporium canis*, *C. glabrata* and *Aspergillus niger* in comparison with miconazole and amphotericin-B. Growth in the medium containing the extract was determined by measuring the linear growth in mm

and percentage growth was calculated with reference to the negative control. The antifungal results are displayed in Table I. The crude extract and its various fractions showed good to excellent activity against *T. longifusus*. *T. longifusus* belongs to the genus Trichophyton, which is the causative agent of dermatophytosis and infects the hair, skin, and nails [22,23]. Trichophyton species may cause invasive infections in immunocompromised hosts [24].

For *T. longifusus*, as shown in Figure 1, crude extract showed (52%) inhibition, while chloroform fraction displayed excellent antifungal activity (67%). Both ethyl acetate and *n*-butanol fractions exhibited significant results, that is, (67%) and (78%) respectively. However, only (18%) antifungal activity was shown by the aqueous fraction. Crude extract and subsequent fractions showed outstanding activity against *M. canis*, which causes the infections of hair, skin and nail. Immunocompromised patients are also infected [25–27]. Crude extract displayed (70%) activity; while chloroform fraction demonstrated outstanding (80%) antifungal activity, ethyl acetate fraction (70%), *n*-butanol fraction (75%) while aqueous fraction was devoted of any activity.

C. albicans and *C. glabrata* were extremely sensitive to *Gloriosa superba* Linn which are the main causative

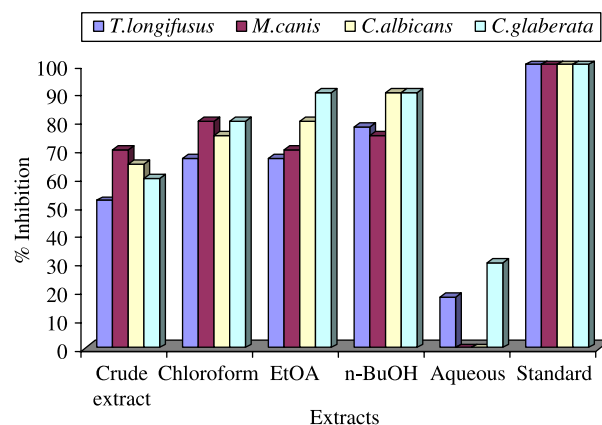


Figure 1. Antifungal activity of the crude extract and subsequent fractions of the *Gloriosa superba* Linn at 400 µg/ mL.

Table II. Antibacterial activities of crude extract and various fractions of *Gloriosa superba* Linn.

Name of Bacteria	Std Drug	Crude extract		Chloroform		Ethyl acetate		<i>n</i> -Butanol		Aqueous	
		ZI	%	ZI	%	ZI	%	ZI	%	ZI	%
<i>E. coli</i>	30	—	—	—	—	—	—	—	—	—	—
<i>B. subtilis</i>	23	3	13%	4	17%	4	17%	7	30%	6	26%
<i>K. pneumoniae</i>	27	—	—	—	—	—	—	—	—	—	—
<i>S. aureus</i>	27	16	59%	14	52%	7	26%	23	88%	—	—
<i>S. flexenari</i>	24	10	42%	08	33%	10	42%	12	50%	11	46%
<i>S. typhi</i>	25	—	—	—	—	—	—	—	—	—	—
<i>Paeruginosa</i>	24	—	—	—	—	—	—	—	—	—	—

Standard drug (Std) = Imipinem, ZI = Zone of inhibition, % = % inhibition.

agents of invasive candidiasis including vaginal candidiasis [28–30]. In case of *C. albicans*, crude extract exhibited (65%), chloroform fraction (75%), ethyl acetate (80%) and *n*-butanol (90%) activity, while aqueous fraction was inactive. For *C. glabrata*, crude extract posses (60%), chloroform fraction (80%), ethyl acetate fraction (90%), *n*-butanol fraction (90%) and aqueous fraction (30%) activity. On the other hand, crude extract and subsequent fractions displayed no anti-fungal activity *A. flavus* and *F. solani* against in the assay.

The crude extract and subsequent fractions of *Gloriosa superba* Linn were also screened against various human pathogens including *E. coli*, *B. subtilis*, *K. pneumoniae*, *S. flexenari*, *S. aureus*, *P. aeruginosa* and *S. typhi*. The results are presented in (Table II). According to antibacterial bioassay, the crude extract and various fractions of *Gloriosa superba* Linn expressed excellent activity against *S. aureus*. The *n*-butanol fraction displayed the highest antibacterial activity (88%) against *S. aureus* in the assay among the tested pathogen. The crude extract exhibited (59%), chloroform fraction (52%), ethyl acetate fraction (26%) while aqueous fraction was inactive in the assay (Figure 2).

The antibacterial activity of the plant was low to moderate in antibacterial bioassay for *S. flexenari*. It has been observed that crude extract exhibited (42%), chloroform fraction (33%), ethyl acetate (42%), *n*-butanol fraction (50%) and aqueous fraction (46%) activity. Plant also exhibited low activity against *B. subtilis*. Crude extract exhibited (13%), while both chloroform and ethyl acetate fraction demonstrated (17%) activity for each and aqueous fraction displayed (26%) activity. The crude extract and subsequent fractions showed no zone of inhibition against, *S. typhi*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

The total phenol content of the crude extract and subsequent fractions of the *Gloriosa superba* Linn were also determined (Figure 3), in order to analyze any possible correlation between the antimicrobial activities and total phenol content of the plant fractions. It is well documented in literature that the phenols have significant antimicrobial activities and have a broad spectrum against both bacteria and fungi [31–34].

In our present investigation, there was no correlation between the antimicrobial activity and phenol content. Therefore, it can be assumed that some other mechanism is responsible for the antifungal and antibacterial effects of this plant.

Results of present investigation, revealed that this plant species has excellent antifungal sensitivity against *C. albicans*, *C. glabrata*, *T. longifusus* and *M. canis* and significant antibacterial activity against *S. aureus*. Therefore, it could be a natural antifungal agent against these pathogens in different infections.

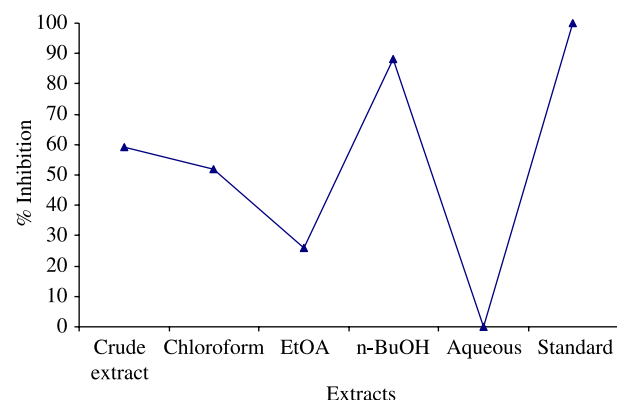


Figure 2. Antibacterial activity of the crude extract and subsequent fractions of the *Gloriosa superba* Linn against *S. aureus* at 100 µg/mL.

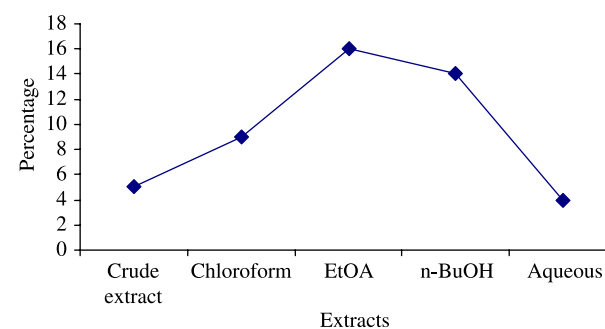


Figure 3. Total phenol content of the crude extract and subsequent fractions of *Gloriosa superba* Linn.

However, further phytochemical and pharmacological investigation of the plant is required to explore the exact mechanism of antimicrobial activity and also to confirm other ethno-botanical uses in indigenous system of medicine.

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