

# Screening of *Bunium bulbocastanum* for antibacterial, antifungal, phytotoxic and haemagglutination activities

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**Abstract:** The current study was aimed at screening the *Bunium bulbocastanum* for its antibacterial, antifungal, phytotoxic and haemagglutination activities. The crude methanolic extract and *n*-hexane fraction showed significant (89%) and good activity (61%) against *Staphylococcus aureus* while the CHCl<sub>3</sub> fraction was moderately active against *S. aureus* (53%). Moderate activity was shown by the EtOAc fraction against *B. subtilis* (44%). This fraction was inactive against *P. aerogenosa* and *S. aureus*. The aqueous fraction showed significant activity against *B. subtilis* (85%), moderate against *S. aureus* (34 %) and *E. coli* (33%) and low activity against *P. aerogenosa* (29%). Our results for antifungal assay indicated that all the test samples were inactive against all the test fungi. The phytotoxic activity of the plant at 1000 and 100 µg/ml was: crude methanolic extract (53.33 and 46.66%), *n*-hexane (46.66 and 26.66%), CHCl<sub>3</sub> (20 and 6.66%), EtOAc (46.66 and 26.66%) and aqueous (40 and 33.33%). All the test samples (crude methanolic extract and fractions) of *B. bulbocastanum* were unable to agglutinate RBCs of the human blood indicating that this species lack phytolectins.

**Keywords:** *Bunium bulbocastanum*, antibacterial, antifungal, phytotoxic, haemagglutination.

## INTRODUCTION

One of the main problems faced by biological sciences in the current era is antibiotic resistance (Heaney *et al.*, 2000; Schmidit, 2004; Klugmandoi, 1990). Among the available antibiotic and fungicides some possess serious side effects like penicillin can cause allergy in certain conditions (Smith 2008; Frigas E 2008; Scott, 2007). On the other hand broad spectrum antibiotics kill the normal flora of the body thereby impairing normal body functions (West *et al.*, 1994; Norrby, 1991). Resistance has also been seen among fungal species. Studies have shown the fungicides; that are used against pathogenic fungal species, also have side effects (Klugmandoi, 1990). Scientists are trying different strategies to find new and innovative antimicrobials agents that can cure diseases without or fewer side effects.

Nature itself is one of the options to find effective antimicrobial drugs (Caotes *et al.*, 2002). Plants have long been used to treat different infections for thousands of years (Kala *et al.*, 2004). Hence there has been a trend to screen different plants for their antibacterial and antifungal properties so we aimed to find out the antibacterial and antifungal properties of *Bunium bulbocastanum*.

The previous studies are convincing evidence that allelopathy has a potential role in natural and agricultural ecosystems (Fujii, *et al.*, 1991). Extensive research has been conducted to find out the phenomenon involved in this mechanism for improving agricultural ecosystems. There has been a continuous effort to use natural products from plants as pesticides rather than synthesizing them in

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laboratories. Among different ways one is to screen plants for their allelopathic potential and to identify plants with phytotoxic capabilities (Fujii, *et al.*, 1991; Fujii, *et al.*, 1990; Fujii, *et al.*, 2003).

At present about 60% of the world's population uses medicinal plants to treat different health problems (Farnsworth, 1994). Of the world 25 best selling pharmaceutical products, 12 are from plants indicating the importance of the medicinal plants in the treatment of diseases (Bashir *et al.*, 2011).

*B. bulbocastanum* (Irani Zeera) is a perennial plant that grows to about 0.6 m (2ft) by 0.3 m (1ft) (Pimenov 1996). It is found primarily in northern India, Kashmir, Afghanistan, Tajikistan and Persia, though they do grow wild as far as South Eastern Europe (Clapham *et al.*, 1962). *B. bulbocastanum* has been used for edible purposes for years. Its seeds can be used either in raw or cooked form to enhance food flavors or to improve its taste (Clapham *et al.*, 1962). Medicinally it is also used as an astringent (Clapham *et al.*, 1962). Keeping in view the novelty of *B. bulbocastanum*, the current study was aimed to screen the fruits of *B. bulbocastanum* for possible biological/pharmacological properties i.e. antibacterial, antifungal, phytotoxic and haemagglutination.

## MATERIALS AND METHODS

### Collection of plant material

The fruits of *B. bulbocastanum* were purchased from board bazar Peshawar, Khyber Pukhtoonkhwa, Pakistan. The samples were kindly identified by Prof. Dr. Abdur-Rashid, plant taxonomist, Department of Botany, University of Peshawar, Pakistan

### Extraction

Whole fruits of the *B. bulbocastanum* were kept in shade for drying. After drying they were grounded to powder using electric grinder. This fruit powder (920 g) was soaked in methanol for 15 days (twice) at room temperature. Each time the mixture was filtered and then the filtrates were combined and concentrated with rotary evaporator at 40°C. A yellowish methanolic extract (137 g) of *B. bulbocastanum* was obtained after concentration.

### Fractionation

The crude methanolic extract of *B. bulbocastanum* (117 g) was suspended in distilled water (350 ml). It was further partitioned with *n*-hexane (3 x 500 ml), Chloroform (CHCl<sub>3</sub>) (3 x 500 ml) and Ethyl acetate (EtOAc) (3 x 500 ml), respectively, to yield the *n*-hexane (37 g), CHCl<sub>3</sub> (25 g), EtOAc (15 g) and aqueous (30 g) fractions. 20 g of the crude methanolic extract of was left for biological /pharmacological activities.

### Antibacterial Activity

Antibacterial activity of the crude methanolic extract and various fractions of *B. bulbocastanum* fruits were determined against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. The method of (Ahmad *et al.*, 2009) was used to determine the antibacterial activity. Eighteen hours old culture of the test organism from the nutrient broth was transferred to sterile nutrient agar plates to make bacterial lawn. Wells were dug in plates using a sterile 6 mm borer after 15 minutes. Stock solutions (3 mg/ml) of the test samples were prepared in sterile dimethyl sulfoxide (DMSO). 100 µl of crude methanolic extract and the fractions were loaded to their respective labeled wells. Amoxicillin and DMSO (less than 1%) were used as positive and negative controls, respectively. Zone of inhibition was measured (in mm) in comparison with positive control. For each sample the procedure was repeated 3 times to achieve higher accuracy.

### Antifungal Activity

Antifungal activity of the crude methanolic extract and various fractions of *B. bulbocastanum* fruits were determined against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum*, *Fusarium oxysporum*, *Trichoderma harzianum* and *Alternaria alternata*. For this activity the reported procedure of Bashir *et al.* (2007) was employed. Test samples (24 mg/ml) were dissolved in the sterile dimethyl sulfoxide (DMSO, Merck), to make stock solution. For each sample the procedure was repeated 3 times. Then to the non-solidified autoclaved, SDA, 67 µl of the test sample was added when its temperature was about 55°C. Tubes were then allowed to solidify in the slanted position at room temperature. Each tube was inoculated with inoculums from a seven days old culture of test fungi. DMSO and standard antifungal drugs served as negative and positive control respectively. Inhibition of

fungal growth was observed after 7 days of incubation at 28±1°C. Results were taken by measuring the linear growth of fungi in slants.

### Phytotoxic activity

The phytotoxic activity of *B. bulbocastanum* test samples (methanolic crude extract and fractions) was determined against *Lemna minor* L by applying the procedure 4 times to each sample. The method of (McLaughlin *et al.*, 1991) was followed. Stock solutions of the test samples were prepared in methanol at concentration of 20 mg/ml. E-medium was prepared for the growth of *L. minor*. 10, 100 and 1000 µg/ml from the stock solution were introduced into three separate flasks and left at room temperature till methanol was evaporated. 20 ml of the E-medium and sixteen healthy plants with a rosette of three fronds were added to all the flasks and incubated at 28 ± 1°C for 7 days. Paraquat at a concentration 0.015 µg/ml was used as standard growth inhibitor. Results were taken by counting the number of damaged and healthy plantlets.

### Haemagglutination activity

The crude methanolic extract and other fractions of *B. bulbocastanum* were screened for possible haemagglutination activity, against human erythrocytes of all blood groups. The method of (Naqvi *et al.*, 1992) was used in this case. Fresh blood was collected from healthy volunteers, centrifuged at 6000 rpm for 10 minutes and the erythrocytes were separated. 2% erythrocytes suspension was prepared in phosphate buffer (pH 7.4). Stock solutions (1 mg/ml) of the test samples were prepared in DMSO and different dilutions (1:2, 1:4, 1:8 and 1:16) were made from it. From each dilution 1 ml was added to 1ml of 2% erythrocytes suspension and incubated at 37°C. For accuracy, each sample investigations were done 3 times. Positive and negative results are indicated by rough granules and smooth button formation, whereas extent of deposition determined the intensity of positive result.

## RESULTS

### Antibacterial activity

*Enterococcus* and *Staphylococcus* species are agents of many nosocomial infections and studies have indicated that they are resistance to available drugs. *Staphylococcus aureus* has become resistant to several antibiotics to which it was previously susceptible. Some of the antibiotics to which *S. aureus* is now resistant are penicillin G, lincosamides, macrolides, tetracyclines, and gentamicin (Ayliffe 1997).

The results for antibacterial study are demonstrated in fig 1. They indicate that the crude methanolic extract showed significant activity against *S. aureus* (89%) and moderate activity against *P. aerogenosa* (59%), *B. subtilis* (48%) and *E. coli* (37%). The *n*-hexane fraction showed good

activity against *S. aureus* (61%) and moderate activity against *E. coli* (48%). It showed low activity against *B. subtilis* (18.5%) and no activity against *P. aerogenosa*.  $\text{CHCl}_3$  fraction was moderately active against *S. aureus* (53%). It showed low activity against *B. subtilis* (22%) and no activity against *P. aerogenosa* and *E. coli*. Moderate activity was shown by the EtOAc fraction against *B. subtilis* (44%) and low activity against *E. coli* (22%). It was inactive against *P. aerogenosa* and *S. aureus*. The aqueous fraction showed significant activity against *B. subtilis* (85%), moderate against *S. aureus* (34%) and *E. coli* (33%) and low activity against *P. aerogenosa* (29%).

The above results indicates that crude methanolic extract have significant activity against *S. aureus* (89%) while the

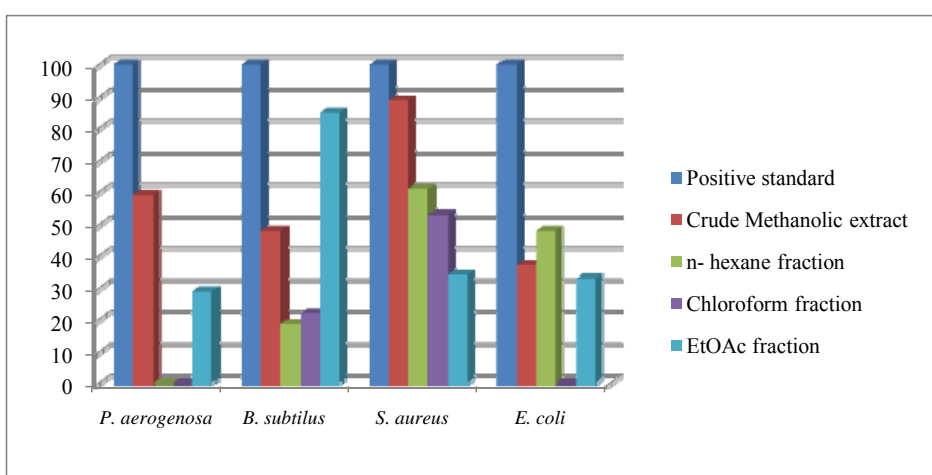
aqueous fraction showed significant activity against *B. subtilis* (85%).

#### Antifungal activity

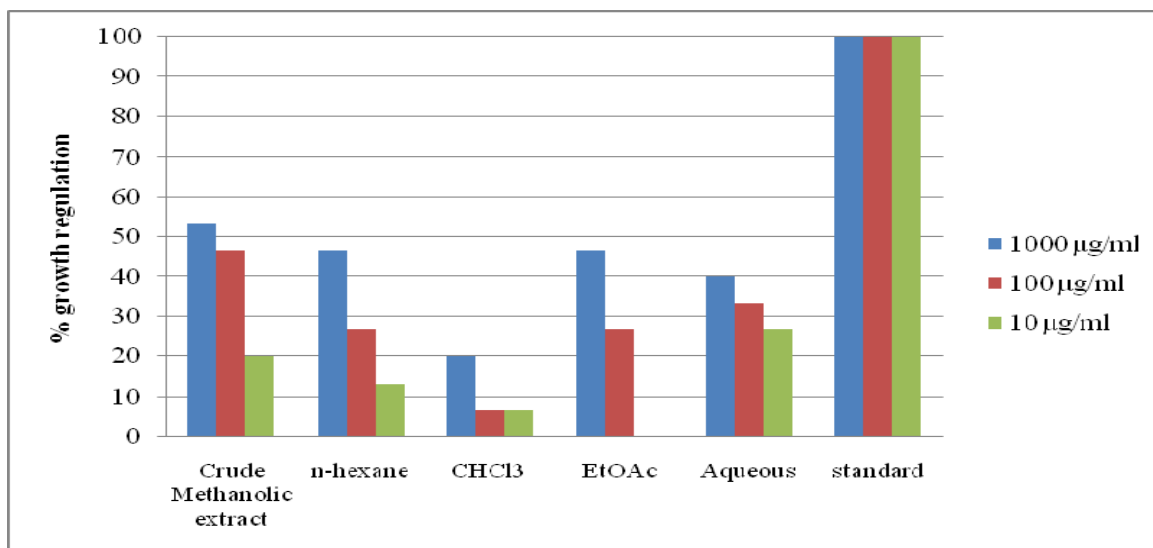
Our results for antifungal assay indicated that the crude methanolic and fractions of *B. bulbocastanum* were incapable of suppressing fungal growth. The results showed that all the test samples were inactive against all the test fungi.

#### Phytotoxic activity

Lemna plants are miniature aquatic monocotyledonous plants that have been used to detect natural phytotoxic and tumor compounds (Rehman1991). Hence it can be used to detect new plant growth inhibitors. Previously, we had also studied the phytotoxicity of the crude methanolic



**Fig. 1:** Antibacterial activity of crude methanolic extract and fractions of *B. bulbocastanum*.



**Fig. 2:** Phytotoxic activity of crude methanolic extract and fractions of *B. bulbocastanum*.

extracts of *Rumex hastatus*, *Rumex dentatus*, *Rumex epalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* (Family *Polygonaceae*) using the Lemna bioassay. The phytotoxic activity of the plant at 1000 and 100 µg/ml was: crude methanolic extract (53.33 and 46.66 %), *n*-hexane (46.66 and 26.66%), CHCl<sub>3</sub> (20 and 6.66 %), EtOAc (46.66 and 26.66 %) and aqueous (40 and 33.33%). At concentration of 10 µg/ml the results (shown in fig 02) were: crude methanolic extract (20%), *n*-hexane (13.33%), CHCl<sub>3</sub> (6.66%), EtOAc (0%) and aqueous (26.66%) respectively.

#### Haemagglutination assay

Lectins are found ubiquitously in plants and other organisms and are able to differentiate different carbohydrates moiety of cell surfaces and in solutions. Using specificity of lectins, studies have been conducted on the structural and functional roles of cell surface carbohydrates (Sharon and Lis 1972). It has also been used to identify sugar components of normal and cancerous cell and for agglutination of erythrocytes to find the blood type and for estimation of the number of virus particles (Wei and Koh 1978). Keeping in view this diverse role of lectins, haemagglutination activity of *B. bulbocastanum* was done against red blood cells (RBCs) of human blood. All the test samples (crude methanolic extract and fractions) of *B. bulbocastanum* were unable to agglutinate RBCs of the human blood indicating that this species lack phytolectins.

#### DISCUSSION

The finding of our previous study for antibacterial activity of the crude methanolic and various fractions of *Acacia modesta* showed that *n*-hexane and EtOAc fractions showed good antibacterial activity against *Klebsiella pneumoniae* (66.66 %) and (61.90%), respectively. All the fractions showed moderate activity against *E. coli* except chloroform (CHCl<sub>3</sub>) (Bashir et al., 2011). Low phytotoxic activity was shown by the test samples at concentration of 1000 and 100 µg/ml against *Lemna minor* L. From current study we conclude that *B. bulbocastanum* has a potential to be used as an antibacterial drug especially its crude methanolic extract against *S. aureus* and presents a good case to be investigated further into. The current study also showed low phytotoxic and no haemagglutination activity. Fungi produce a number of secondary metabolites (mycotoxins) and some have been found to be toxic to humans and animals for example *A. niger* produce Malformins. (Smedsgaard., 1997). Previously we carried out the antifungal activity of the crude methanolic extract and various fractions of *A. modesta* against *A. niger*, *A. flavus*, *P. notatum*, *F. oxysporum*, *T. harzianum* and *Rhizopus stolonifer*. Only chloroform (CHCl<sub>3</sub>) fraction exhibited low activity (15%) against *F. oxysporum* (Bashir et al., 2011)

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