



Antifungal Activity in *Urginea indica* Kunth. (Asparagaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author BM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SK and MKPK designed the study and approved the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aqueous extract of *Urginea indica kunth*. (Udupi accession) was screened for antifungal activity against *Fusarium oxysporum*, *Sclerotium rolfsii*, *Magnaporthe oryzae*, *Aspergillus flavus*, *Alternaria alternata*, *Aspergillus niger* and *Fusarium moniliformae* by poisoned food technique. The results confirmed *Urginea indica* extracts showed very significant antifungal activity against *Fusarium oxysporum* and showed significant inhibition for *Sclerotium rolfsii* and *Magnaporthe oryzae* it showed no activity against *Aspergillus niger* and *Aspergillus flavus*. All the activity was evaluated to determine the lowest concentration required to inhibit visible mycelial growth of the pathogen at minimum concentration. *Fusarium oxysporum* showed very significant inhibition in 10% concentration (Reconfirmed) while *Sclerotium* showed significant inhibition in 25% concentration followed by *Magnaporthe oryzae*. The number of sclerotia spores formed was also reduced drastically. These results show that a potential and safe antifungal agent can be obtained from *Urginea*.

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1. INTRODUCTION

Urginea indica is also known as Indian squill, commonly called as wild onions. It is a member of Asparagaceae family. The Squill bulb has long been used as a source of medicinal product with pharmaceutical and biocidal applications [1]. Medicinal plants constitute a very important natural resource used by indigenous medicinal systems for the past 300 years [2]. *Urginea* is one of the extremely interesting polytypic genera with about 100 species and is represented in India by nine species [3]. Bufadienolides of the squill are glycosides used as cardio-tonic agents. The toxicity of the squill bulb is attributed to its scilliroside content [4-10]. A significant portion of the agricultural product in the country and the world wide has become unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of *Aspergillus*, *Fusarium* and *Penicillium* [11]. The annual crop losses of world as a result of diseases have been estimated at 25,000 million US dollars, of this a major part is due to fungal pathogens carried through seed (Agrios, 1997).

Fungi are significant destroyers of foodstuffs and grains during storage, rendering them unfit for human consumption by retarding their nutritive value and often by producing mycotoxins [12]. Eventhough effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity [13]. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails [11]. In folk medicine, medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases [8]. In recent years there has been a revival of interest in the use of medicinal and aromatic plants in developed and developing countries [14,15]. The plants world is a rich storehouse of natural chemicals that could be exploited for use as pesticides. The total number of plant chemicals may exceed 4,000,000 and of these 10,000 are reported to be found secondary metabolites whose play a major role in the plants is reportedly defensive [16]. In view of these, the present investigation was undertaken to screen for the efficacy of antifungal potency of *Urginea indica* plant extracts against important phytopathogenic fungi.

2. MATERIALS AND METHODS

Collection of plant material: The bulbs of *U. indica* were collected from Udupi, Karnataka. The collected materials were authenticated and the voucher specimens are preserved. The bulbs collected across were labelled and maintained in the germ plasm established in the Department of Botany, Bangalore University. In the present study *Urginea* bulbs were collected from Annegudde (Udupi) were used to study antifungal activity.

Preparation of aqueous extract: The freshly collected bulbs were washed in running tap water and then rinsed in distilled water. The bulbs were shade dried and pulverized using an aseptic electric blender to obtain a powdered form and it was sieved and stored in sterile polyethylene sample bags prior to use [17,6]. 2 gms of the sample was macerated with 14 ml of sterile water, (1:7 w/v) (modified sateesh 1998) the sample was mecerated in a mortar and pestle for 10 minutes, filtered using double layered muslin cloth. The filtrate was centrifuged at 5,000 RPM for 20 minutes at 4° and supernatant was filtered through Whatman no. 1 filter paper and was considered as 100% and further dilutions of 75%, 50%, 25% were prepared accordingly and subjected to antifungal assay.

Test organism: The test fungi *Aspergillus niger* and *Aspergillus flavus*, *Rhizopus stolonifer* were isolated through seed blotter technique, while *Phomopsis azadirachatae* was obtained from Department of Microbiology and plant Biotechnology, Bangalore University. The remaining test fungus were obtained from GKVK University pathology department.

Isolation of phytopathogenic fungi was done by using seed blotter technique using Glycine max (soybean) seeds. Soybean seed samples were surface disinfected with Sodium hypochlorite (1%) solution for about 2 min at room temperature and placed in Petri plates (10 seeds/plate). The plates were subjected to SBM ISTA (1996). On the seventh day incubation, seeds were observed under stereo binocular microscope. *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alta*, *Rhizopus* species, was identified based on their growth, mycelial structure and spore morphology using standard manuals [18]. through seed blotter technique. The isolated pathogen was sub cultured onto PDA and maintained at 4°C for further use. *Sclerotium*

rolfsii, *Magnaporthe* fungal cultures were obtained from plant pathology Department, GKVK college, Bangalore.

Antifungal assay: The Fungus was sub cultured on PDA medium amended with 100 µg/ml streptomycin, at 25°C for ten days near bright light. Five mm diameter mycelial disc retrieved from fresh cultures grown on PDA plates served as fungal inoculum [18]. The antifungal activity of the crude extracts of *Urginea indica* plant bulb was evaluated by poisoned food technique using the Potato Dextrose Agar (PDA) medium.

Poisoned food method: *Urginea indica* bulb extract is incorporated into the molten agar at a desired final concentration and mixed well (25%, 50%, 75% and 100%). After overnight pre-incubation, 15 ml of the medium was poured into each petriplate, allowed to cool and solidify, Five mm mycelial disc from seven-day old culture of the test culture and was inoculated aseptically at the center of each plate and incubated at 25 ± 20°C for ten days near bright light. The Culture grown on PDA plates without plant extract served as control and PDA plates amended with bavistin (1 mg/ml) served as positive control. Percentage inhibition of mycelial growth, the diameters of fungal growth in control and sample plates are measured, Each experiment was repeated three times and the antifungal effect was estimated by the following formula:

$$\text{Antifungal activity (\%)} = ((D_c - D_s) / D_c) \times 100$$

Where D_c is the diameter of growth in control plate and D_s is the diameter of growth in the plate containing tested antifungal agent.

3. RESULTS AND DISCUSSION

In the present study, crude aqueous extracts of *Urginea indica* was evaluated for antifungal activity against some important phytopathogens by poisoned food technique. Variable level of inhibition was exhibited by different herbal

extracts. Out of the phytopathogens screened, *Sclerotium rolfsii*, *Magnaporthe oryzae*, *Phomopsis azadirachtae* showed significant inhibition than control (Table 1). The plant extracts which exhibited 100% inhibition were further evaluated to determine the least percentage required for complete inhibition of the pathogen. The minimum percentage required to inhibit visible growth of the pathogen was found to be 2% for *Fusarium oxysporum*, 25% for *Sclerotium rolfsii* 30% for *Magnaporthe oryzae* showed complete inhibition. Rest showed little or no inhibition.

There is a large demand for new fungicides for use in food protection, agriculture and medicine. In recent years there has been a growing trend to evaluate the antimicrobial activity of the extracts and isolates of medicinal plants, because of resistance developed by pathogens [3,15]. Even though effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity. Extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails [8]. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides [2]. Antifungal experiments carried out the antifungal activities of *Urginea indica*. A novel glycoprotein called 29 KDa was isolated from it. 29 kDa glycoprotein suggests that the glycan part of the molecule appears to be involved in antifungal activity. Antifungal tests have demonstrated that *Urginea indica* protein exerts a fungistatic effect. It completely inhibits the germination of spores and hyphal growth of *Fusarium oxysporum*. In continuation of this, the present investigation was undertaken to screen for the efficacy of antifungal potency of *Urginea* bulb extract against other important phytopathogenic fungi.

Table 1. Antifungal activity of *Urginea indica* extract against some phytopathogenic fungi (Percentage of mycelial inhibition)

Sl No	Test fungus	25%	50%	75%	100%	Control	% inhibition
1	<i>Sclerotium rolfsii</i>	6.1	5.6	5.4	4.6	9.3 cms	50.53%
2	<i>Magnaporthe oryzae</i>	-	-	-	-	9.1 cms	100%
3	<i>Phomopsis azadirachtae</i>	8.6	8.0	7.9	7.1	8.9 cms	25.32%
4	<i>Aspergillus flavus</i>	9.2	9.3	8.9	8.6	9 cms	-
-	<i>Aspergillus niger</i>	8.8	8.6	8.0	8.0	8.9	-
6	<i>Alternaria alternata</i>	8.9	9.1	8.5	8.8	8.6	-
7	<i>Fusarium moniliformae</i>	7.6	8.3	8.1	7.9	7.8	-

Antifungal index are given as mean of three replicates

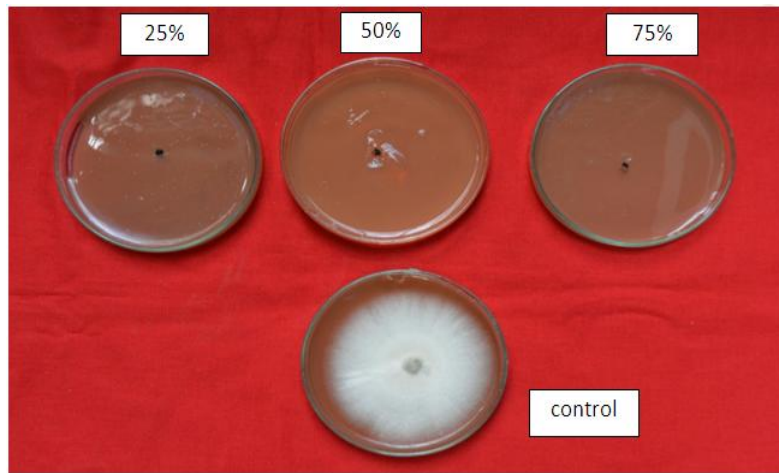


Fig. 1. Showing inhibitory effect of different concentrations on *Magnaporthe oryzae*



Fig. 2. Showing mycelial inhibition of *U. indica* bulb against *Sclerotium rolfsii*



Fig. 3. Showing mycelial inhibition of *U. indica* bulb against *Phomopsis azadirachtae*

4. CONCLUSION

U. indica is a bulb called as Indian squill with rich medicinal attributes. It is found growing in amidst rocks and grasses, in sandy soil, in the foot hills of Himalayas.

Thus, *Urginea* occurs in xerophytic and mesophytic habitats. During the course of screening *U. indica* bulb for antifungal activity, we found that it exhibited antifungal activity against many. The extract of *U. indica* bulb shows more effect when not autoclaved. It indicates that the compound responsible for antifungal activity may be a protein. Several active compounds from *U. indica* bulbs such as 29 KDA protein. This 29KDA –endochitinase might have played role in inhibiting the growth of agronomically important pathogenic fungi such

as *Magnoporthae*, *Phomopsis* and *Sclerotium rolfsii*. Therefore, further detailed study on *U. indica* phytochemistry is needed for isolation and purification of secondary metabolites which may further yield significant antifungal agents against agronomically important fungus. Thus there is a need to search for alternative approaches to store grains for human consumption without toxicity problems that are ecofriendly and not capital intensive. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. Plants with their complex chemical store house of biodynamic compounds, serve as a plant defence mechanisms against invasion of microorganisms, and provide a natural source of antimicrobial agents. The active principles isolated from plants appear as an important alternative than synthesized drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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