Original Article

Screening of Fungicidal Activity of *Salix* and *Triumfetta* Species of Garhwal Himalaya

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ABSTRACT

The aim of present study is to find out fungicidal activity of *salix babylonica* and *triumfetta pillosa*. Plant extract was mixed in sterilized distilled water to make the desired concentration (v/v) for experiments. Poisoned food technique was used to screen different plant extracts *in vitro*. Different concentrations (2, 5, 10, and 20%) of plant extracts were incorporated to Oat meal Agar medium for inoculation of the test pathogen in sterilized petridishes. The isolated pathogen grown on Oat meal agar medium was placed at the center of petridishes containing different concentration of the poisoned medium and incubated at 28±1°C for 8 days. Radial growth (cm) of fungus was measured after inoculation till 8 days at an interval of 24 hours

Keywords: Fungicidal, *salix* and *triumfetta* species, Garhwal Himalaya, *Fusarium oxysporum*.

INTRODUCTION

The prevalence of resistance to antifungal agents significantly increased in the past decade. Resistance to antifungal agents has important implications for morbidity, mortality and health care in the community. Until recently, fungi were not recognized as important pathogens because the annual death rate due to candidiasis was steady from 1950 to 1970[1,2] Since 1970, this rate increased significantly due to more widespread use of immunosuppressive therapies, indiscriminate use of broad-spectrum antibacterial agents, the common use of indwelling intravenous devices and immuno suppressive viral infections such as AIDS. These developments and the associated increase in fungal infections[3] necessitated the search for new, safer, and more potent agents to combat serious fungal infections. For nearly 30 years, amphotericin B, which causes significant nephrotoxicity,was the sole drug available to treat serious fungal infections. The imidazoles and the triazoles in late 1980s and early 1990s were major advances in safe and effective treatment of local and systemic fungal infections. The high safety profile of triazoles, in particular fluconazole, has led to their extensive use. Fluconazole has been used to treat in excess of 16 million patients,

including over 300,000 AIDS patients, in the United States alone since the launch of this drug[4]. Due to selective pressure and widespread use of these few antifungal drugs, there have been increasing reports of antifungal resistance[5]. Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. Relatively 1-10% of plants are used by humans out of estimated 250,000 to 500,000 species of plants on Earth[6]. The plants are relatively cheap source of biological material having a vast variety of metabolites, primary and secondary, available in them for selecting the molecule of desired biological activity. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years is due to the rapid extinction rate of (plant) species[7]. The scientific discipline, ethno botany, is utilizing the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health[8,9]. Lastly, the ascendancy of the human immunodeficiency virus (HIV) has spurred intensive investigation into the plant derivatives, which may be effective, especially for use in under developed nations. Few of the compounds isolated from plants such as 2-decanone, hydroxydihydrocornin-aglycones9, various indole derivatives[10] and isoflavanone are reported to have antifungal activities. However, development of useful antifungal drugs from these compounds has not yet been possible. The fruit juice of the plant *Triumfetta pillosa* is applied on cuts, its fruit infusion is given to women to facilitate delivery[11]. The ethanolic extract of the rhizome of the plant showed significant antifungal activity. The ethanolic extract of the roots were analyzed for anticandidala activity[12].

The purpose of this study was to investigate experimentally the possible fungicidal activity of *S.babylonica* (roots) *and T.Pillosa* (whole plant) against *Fusarium oxysporum.*

EXTRACT PREPARATION

The plants were dried at room temperature under shade and later grounded into fine powder. Ethanolic extract was prepared by putting plant material (250g) in soxlet apparatus with 95% EtOH. The extract was filtered using a buckner funnel and whatman no.1 filter paper. The filtrate was evaporated until it becomes thick paste.

PREPARATION OF BOTANICAL CONCENTRATION

The appropriate amount of plant extract was mixed in sterilized distilled water to make the desired concentration (v/v) for experiments. For bioassay, double strength concentrations of botanicals were prepared by dissolving 2.5, 5, 10 and 15ml of plant extract in 90, 80, 70 and 60ml of sterilized distilled water respectively to get the final concentration of 2.5, 5, 10 and 15%.

BIOASSAY PROCEDURE FOR ANTIFUNGAL ACTIVITY

Poisoned food technique was used to screen different plant extracts *in vitro.* Different concentrations (2, 5, 10, and 20%) of plant extracts were incorporated to Oat meal Agar medium for inoculation of the *Fusarium oxysporum* in sterilized petridishes. The isolated pathogen grown on Oat meal agar medium was placed at the center of petridishes containing different concentration of the poisoned medium and incubated at 28±1°C for 8 days. Radial growth (cm) of

 ISSN 2321 – 2748

fungus was measured after inoculation till 8 days at an interval of 24 hours.

The inhibitory effect was worked out by using following formula:

Percent inhibition = $\frac{x - y}{x} \times 100$

Where,
x = Fungal colony diameter in control
y = Fungal colony diameter in treatment

The preliminary phytochemical screening was performed for the ethyl acetate fraction of EESS[18-20].

RESULTS AND DISCUSSION

The two plant extract used to determine fungal activity. Ethanolic extract of *Salix babylonica* showed a good activity with 20% concentration (Table 1), ethanolic extract of *Triumfetta pillosa* (Table 2) showed a weak activity against *Fusarium oxysporum*.

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 ISSN 2321 – 2748

Table 1. Effect of plant extract on the radial growth of *Fusarium oxysporum*

Concentration (%)	Radial growth of fungus (cm)	Control	Growth inhabitation (%)
2.00	7.95	9.00	12.00
5.00	7.62	9.00	15.33
10.00	7.60	9.00	16.00
20.00	6.37	9.00	29.22

Table 2.

Concentration (%)	Radial growth of fungus (cm)	Control	Growth inhabitation (%)
2.00	8.40	9.00	7.00
5.00	8.32	9.00	7.55
10.00	8.29	9.00	8.00
20.00	8.02	9.00	11.00