Study of antifungal activities of *Shorea robusta* Gaertn against *Fusarium oxyporum*

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Abstract: **The present investigation has been done using aqueous and ethanol extract of Shorea robusta Gaertn against *Fusarium oxyporum* MTCC-284 strain. Bark, leaves, flowers and fruits as plant organs were used for plant extracts. Phytochemical tests and antifungal tests were performed. Sabouraud Dextrose Agar as a medium used to perform agar well diffusion technique. 250mg/ml concentration of extract was used. One dilution that is 31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml and 3.91 mg/ml were used where 31.25 mg/ml concentration results best for ethanolic extracts against *Fusarium oxyporum*.**

***Keywords*: Plant Extracts, *Shorea robusta*, Antifungal activities, *Fusarium oxyporum*, Phytochemicals analysis.**

Introduction

Plants have been involved as major source of medicines since, ancient era performing a tremendous service to human as well as for plants. Many of which, have been identified, and remains still too to discover, whether as ayurvedic medicines or bio-products, which show effective values. Fusarium vascular wilts and Fusarium root and crown rots are some of the most widespread and destructive diseases of many horticultural crops[1]. Eco-friendly methods involving plant products and biological agents, which act directly on the pathogens or indirectly by inducing resistance in plants, have gained considerable importance as an alternative to using synthetic fungicides[2]. Controlling *Fusarium* wilt is very difficult because it spreads so fast and it is estimated that nearly 80% of the crop damage worldwide is caused due to this busy fungi[3]. Plant extracts have proved to be complementary control means for soil-borne pathogens[4][5]. Natural products are very effective solution to the environmental problems caused by the synthetic fungicides and many investigators are trying to know the effective natural products to replace the synthetic pesticides[6]. Plant metabolites and plant based pesticides are considered to be another alternative as they are known to have minimal environmental impact and minimal danger to consumers in contrast to synthetic pesticides[7]. Use of natural products for the control of fungal diseases in plants is considered as an alternative to synthetic fungicides, due to their lower negative impacts on the environment[8]. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and nonphytotoxic, unlike chemical fungicides[9][10][11].
In this study, *Shorea robusta* Gaertn, commonly called 'Sal' is abundant in Jharkhand state of India opening an opportunity to discover the medicinal properties and hence, proceeded for antifungal studies.

Materials and methods

Plant organs as leaves, flowers, fruits and barks of *Shorea robusta* Gaertn were collected from the Ranchi district of Jharkhand. Organs were separately dried in the dark place and converted to fine powders by mixer-grinder and lastly by sieve. 5 gm fine powders were diluted in the 100 ml solvents and kept overnight. Solvents as ethanol and aqueous were used to collect the two different extracts of each of the samples. Then the supernatant was filtered by 0.22 µm sterile filters (Millipore, Bedford, Massachusetts, USA) and stored at 4 °C until further use. The qualitative detection of phytochemicals was tested by the standard methods. The Phytochemical tests included alkaloids, flavonoids, glycosides, phenols, reducing sugar, tannins, terpenoids and steroids. Antifungal tests were performed by Agar well diffusion Method. The media used for antifungal test was Sabouraud Dextrose Agar (SDA). 32.5 gm of SDA powder was suspended in 500 ml of distilled water. Then, it was boiled to dissolve the medium completely. The SDA media was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and cooled to approximate temperature 45ºC - 50°C. Finally, mixed well and poured into sterile Petri plates. After solidification, wells of 8 mm diameter were punched into the agar medium and filled with 100 µl (250 mg/ml) of plant extracts. Inoculums containing 106 cfu/ml of fungal culture were spread on SDA plates with a sterile swab moistened with the fungi. The plates were then incubated in the upright position at 28° for 72 hours. Wells containing the same volume of ethanol and distilled water served as solvent controls while standard antibiotic discs of Amphotericin B (20 µg, HiMedia, Mumbai, India) were used as the positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm. Subsequently, the method was repeated with four different concentration of extracts consecutively reduced to half as 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml.

Result and Discussion

Phenols are responsible for antifungal activity against *Fusarium oxyporum*[12]. Detection of terpenoids may responsible for mycelial inhibition. Thus, mycelial growth inhibition of monoterpenoids might be caused by complex functions such as biodegradation and/or detoxification by interaction between mycelium and compound[13]. The antifungal activity of flavonoids which represent a potential alternative to conventional fungicides is presented[14]. Except alkaloids, glycosides,steroids, tannins and reducing sugar also detected.

Table 1: Detection of Phytochemicals by standard tests

Sl. No.	Phytochemicals	Observations
1	Alkaloids	Negative
2	Flavonoids	Positive
3	Glycosides	Positive
4	Phenols	Positive
5	Reducing Sugar	Positive
6	Steroids	Positive
7	Tannins	Positive
8	Terpenoids	Positive

Many agriculturally important pesticides have been banned by World Health Organization (WHO) due to their wide range of toxicity against non target organisms including humans, besides such pesticides are also known to cause pollution problems[15]. The use of plant extracts in the treatment of diseases caused by bacteria, viruses and fungi have already been reported. Fungitoxic properties of plant extracts are widely recognized[16][17][18]. An exiguous activity was noted in aqueous extracts of barks, leaves, flowers and fruits of *Shorea robusta* against *Fusarium oxysporum* as revealed in the table 1. All the aqueous extracts show a gradual decrease in zone of inhibition as their respective concentrations reduced. Conclusively, all 4 aqueous extracts did not show anti-fungal activity against *Fusarium oxysporum*, as 5 mm Zone of Inhibition was considered antifungal.

Table 2:
Zone of inhibition in millimeters (mm) of plant organs of *shorea robusta* in aqueous and ethanol extracts against *fusarium oxyporum*. (BA-barks, LE-leaves, FL-flowers and FR-fruits)

Concentration (mg/ml)	Aqueous Zone of Inhibition(mm)				Ethanol Zone of Inhibition(mm)				Positive Control Zone of Inhibition(mm)
	BA	LE	FL	FR	BA	LE	FL	FR	Amphotericin B
250 mg/ml	3	2	3	4	19	20	21	20	21
125 mg/ml	2	1	2	2	11	11	10	11	21
62.5 mg/ml	1	0	1	1	8	8	8	7	21
31.25 mg/ml	0	0	0	0	8	6	8	6	21

While, strong antifungal activity potential were observed in all the 4 alcoholic extracts of barks, leaves, flowers and fruits of *Shorea robusta* against *Fusarium oxyporum*. Ethanolic extracts flowers are almost equal to the positive control whereas, ethanolic extracts of leaves and flowers shows 1 mm less while ethanolic extracts of barks shows only 2 mm less in zone of inhibition. Based on initial screening, by well diffusion method at maximum dose (250 mg/ml), the table2 revealed the antifungal activity of ethanolic extracts of plant organs barks, leaves, flowers and fruits of *Shorea robusta* were evaluated, for dose dependent activity based on Minimum Inhibition Concentration in SDA media (31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml and 3.91 mg/ml) that was consecutively reduced to half.

Table 3:
Minimum inhibitory concentration (mic) zone of inhibition in millimeters (mm) of plant organs of *shorea robusta* in aqueous and ethanol extracts against *fusarium oxyporum*.(BA-barks, LE-leaves, FL-flowers and FR-fruits)

Concentration (mg/ml)	Ethanol Zone of Inhibition(mm)				Positive Control Zone of Inhibition(mm)
	BA	LE	FL	FR	Amphotericin B
31.25 mg/ml	8	6	8	6	21
15.62 mg/ml	4	2	3	3	21
7.81 mg/ml	2	1	2	1	21
3.91 mg/ml	1	0	0	0	21

Best antifungal results were observed for the ethanolic extracts of the plant organs bark, leaves, flowers and fruits of *Shorea robusta* at 31.25 mg/ml concentration, against *Fusarium oxysporum*.

Conclusion

Antifungal activity has been confirmed in plant organs of Shorea robusta Gaertn against Fusarium oxyporum. Occurrence as natural fungicide, possibly in the market as a bio-product and will be vital for crops or vegetables.

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