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ANTIFUNGAL ACTIVITIES OF DIFFERENT DEVELOPMENTAL GROWTH STAGES OF *CASSIA FISTULA* L.

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ABSTRACT

The different developmental growth stages of pod and stem bark of *Cassia fistula* were screened for the antifungal activity against two pathogenic microorganisms by agar well diffusion method. Aqueous, ethanol and benzene extracts from the different developmental growth stages of pods of *Cassia fistula* were tested against fungi. *Cassia fistula* used in Ayurvedic medicine. This plant is used in the treatment of skin diseases, inflammatory diseases rheumatism and anorexia. Antifungal activities of various extracts were compared with commercially available antibiotic. The result revealed that aqueous extract and ethanolic extract showed maximum antifungal activity. Thus both extracts was found to be fungi static and it can be used in drug development to control various fungal infection.

KEYWORDS: Antifungal activity, microorganism, pesticides, Cassia fistula, Antimicrobial activity, Medicinal plant.

INTRODUCTION

Plant pathogens such as viruses, bacteria, fungi diseases in plants.[1] Out of this, fungi pathogens that damages the plant. The application of various natural medicines with their bioactive compound as an alternative medicines develops the immunity. Last few decades, traditional medicines are used to cure various diseases. Up till date 119 drugs were extracted from the plants. Plants are a primary source of new natural medicinal product.[2] In vitro it is found that, flavonoids, tannins, alkaloids, glycosides, terpenoids etc. secondary metabolites are abundantly present in plants which showed antimicrobial properties.[1,3] The various plant parts of *Cassia fistula* are used by various tribal to treat various ailments including ringworm and other fungal skin infection.[4] Indian people use fruit as anti-inflammatory, antipyretic, antimicrobial, purgative, refrigerant and good for chest complaints, eye ailments, heart and liver ailments and rheumatism.

C. fistula is small, deciduous medium sized[5] ornamental[6] tree. In different countries like Mauritius, India, Nepal, China, South Africa, East Africa, West Indies, Brazil and Mexico. *C. fistula* L. is grown extensively[7,8,9] as an ornamental tree for its lovely lots of yellow blossom. The stem bark of *C. fistula* is laxative, antitubercular, anthelmintic, emetic, febrifuge, diuretic, constipation, fever, diabetic, and cardiac problem.[10] In Ayurvedic medicines *C. fistula* has been used against various disorders such as diabetes pruritus, haematemesis, leucoderma, and other ailments.[11, 12, 13] In ancient India, *C. fistula* has been used in the treatment of various ailments, dated back to Sushruta Samhita and Charaka Samhita.[14] The whole plant is used in the treatment of skin diseases, inflammatory diseases rheumatism, anorexia.[15,16]

In this investigation, antifungal activity of different developmental growth stages such as one month pod. 4 month pod, pulp, young stem bark and old stem bark are studied against some pathogen.

MATERIALS AND METHODS

i. Preparation of extract

The different developmental growth stages of pod and stem bark of *Cassia fistula* were collected and dried in oven. The dried material was powdered. 5 gram dried powder was soaked in 50ml each of distilled water, ethanol and Benzene separately at room temperature. The soaked material was shaking for 48 hours on shaker and filtered through Whatman No. 1 filter paper. Obtained filtrate was condensed on water bath up till dry and then made total volume 25 ml and used for further study. The samples were prepared in respective solvent (200mg/ml)

ii. Preparation of Media

Take 200 grams of potato and cut into small pieces by removing outer cover. Boil it into 700ml distilled water. Cool and pour liquid potato extract through muslin cloth. Add 20gm dextrose and 20gm agar-agar with 300ml distilled water. Test the pH of the solution with pH

paper. Thoroughly mix the medium and pour into conical flask. For sterilization keep in autoclave for half an hour at 15lbs pressure. After cooling the flask, pour media in petriplate. The plates were allowed to cool at room temperature to solidify the medium.

iii. Microorganism used

The fungal culture *Aspergillus* and *Fusarium spp.*were obtained from Department of Microbiology, Shivaji university, Kolhapur. The cultures were subcultured periodically and used for antifungal study.

iv. Determination of antifungal activity by agar well diffusion method

To determine antifungal activity agar well diffusion method was employed (Perez et al., 1990).[17] With the help of sterilized cork borer, well of 10mm diameter was prepared. Standard antibiotic Albendezol 100 µg/ml was served as positive control and ethanol, benzene and distilled water as negative control. The plates inoculated with different fungal species were incubated at 37^0C in incubator for 72 hours. Zone of inhibition was measured (diameter in mm). For each treatment three replicates were maintained.

RESULT AND DISCUSSION

In all over world antimicrobial properties of medicinal plants are increasing. By traditional therapies 80% of the world's population are used the plant extract to cure disease. In the present investigation, the minimum inhibition concentration (MIC) of aqueous, ethanolic and benzene extract of different developmental stages of stem bark and pods of *C. fistula* against *Fusarium* and *Aspergillus* is shown in **Table No.1. to 6 and plate No. 1 to 4.** The effect of aqueous pod and stem bark extract of *C. fistula* on the growth of fungi such as *Aspergillus* and *Fusarium* is shown in **Table No.1.** It is observed from table that minimum inhibition concentration of stem bark 5mg/ml is 7mm against *Fusarium* in aqueous extract of 1month pod. From the table it is noticed that the pulp and young stem bark displays maximum zone of inhibition in aqueous extract against *Fusarium.* The effect of ethanolic pod and stem bark extract of *C. fistula* on the growth of microorganism is shown in **Table No. 3.** It is observed that ethanolic 1 month pod and pulp showed maximum zone of inhibition against *Fusarium.* 4 month pod and young stembark showed minimum zone of inhibition against fusarium. Young stembark shows maximum zone of inhibition against *Aspergillus.*

The effect of benzene pod and stem bark of *C. fistula* on the growth of microorganism is shown in **Table No. 5.** From the table, it is observed that, 4 month pod and old stembark showed maximum zone of inhibition against *Fusarium.* One month pod shows minimum zone of inhibition against *Aspergillus.*

CONCLUSION

From the present investigation, it has been concluded that the effect of aqueous pod and stem bark extract of *C. fistula* on the growth of fungi such as *Aspergillus* and *Fusarium* was showed zone of inhibition of stem bark 5mg/ml is 7mm against *Fusarium* in aqueous extract of 1month pod. It is noticed that the pulp and young stem bark displays maximum zone of inhibition in aqueous extract against *Fusarium.* The ethanolic 1 month old pod and pulp showed maximum zone of inhibition against *Fusarium.* 4 month old pod and young stem bark showed minimum zone of inhibition against *Fusarium*. Young stem bark showed maximum zone of inhibition against *Aspergillus.* The 4 month old pod and old stem bark showed maximum zone of inhibition against *Fusarium.* One month old pod shows minimum zone of inhibition against *Aspergillus.*

Plate -1

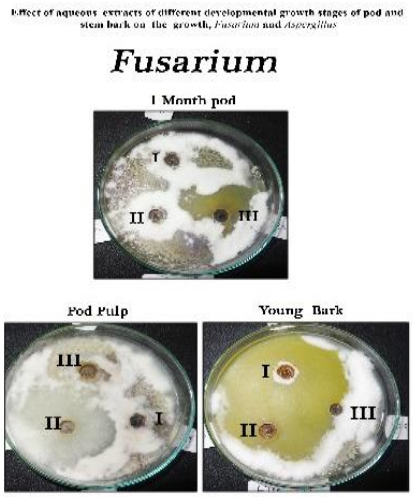

Plate -2

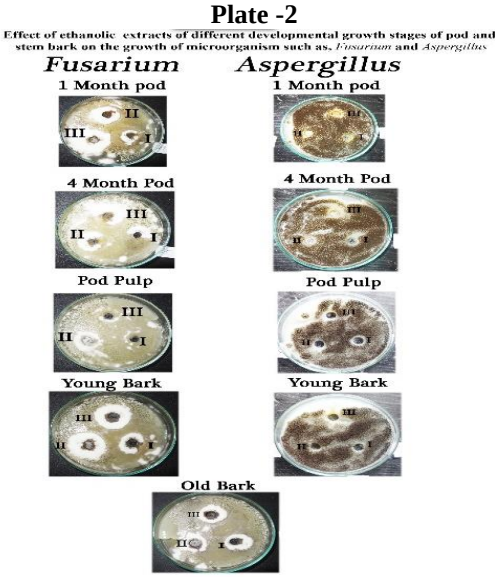

Plate -3

Effect of benzene extracts of different developmental growth stages of pod and stem bark on the growth of, *Fusarium* and *Aspergillus*

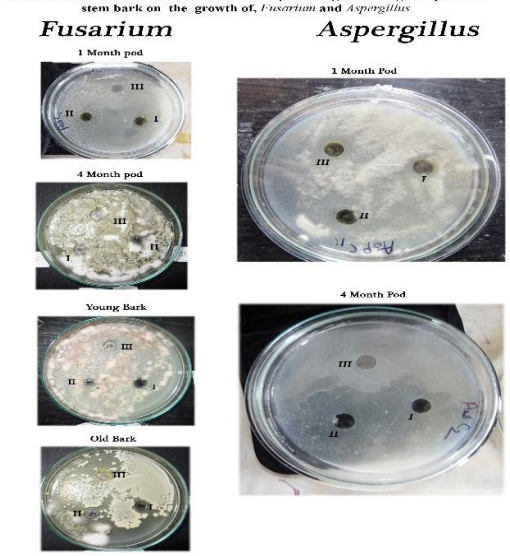

Plate -4

Effect of Aqueous, Ethanol, Benzene extract and Albendezol on the growth of *Aspergillus* and *Fusarium*

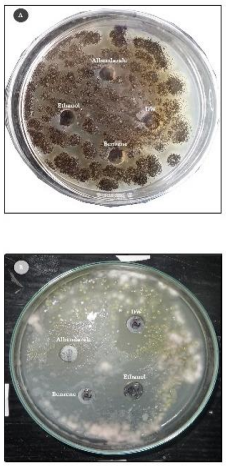

Table No. 1: Minimum inhibition concentration of aqueous extract of pod and stem bark of *C. fistula* against *Fusarium, Aspergillus.*

	Diameter of zone of inhibition in mm																
	1month pod (B1)			4 month pod (B2)			Pulp (B3)			Young bark (B4)			Old bark (B5)			Albendazole	DW
Conc. in ml	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20	1	
Name of pathogen																	
Fusarium	-	-	-	-	-	-	8.16± 1.04	10.5± 1.32	14± 1.7	10.33± 3.21	9.33± 1.15	12± 2.64	-	-	-	16± 0.57	-
Aspergillus	-	-	-	-	-	-	13± 0	12± 0	13± 0	-	-	-	-	-	-	18± 0.27	-

Table 2: Effect of aqueous extract of different developmental growth stages of pod and stem bark *C. fisula* on the growth of against *Fusarium, Aspergillus.*

Sr. No.	Name of the plant part	Name of organisms	
		Fusarium	Aspergillus
1	1Monthpod	-	
2	4 Month pod	-	
3	Pulp	14±1.73	13±0
4	Young stem bark	-	-
5	Old stem bark	12±2.64	-
6	Albendazole	16±057	18±0.27
7	D.W.	-	--

Table No. 3: Minimum inhibition concentration of ethanolic extract of pod and stem bark of *C. fistula* against *Fusarium* and *Aspergillus.*

	Diameter of zone of inhibition in mm																
	1month pod (B1)			4 month pod (B2)			Pulp (B3)			Young bark (B4)			Old bark (B5)			Albendazole	ethanol
Conc. in ml	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20		
Name of pathogen																	
Fusarium	10.6± 1.15	10.33 ± 0.57	10.5 ± 2.59	6.33 ± 1.52	7.33 ± 0.57	7 ± 0	8.33 ± 4.04	7 ± 0	8 ± 0	7 ± 0	7 ± 0	7 ± 0	9 ± 0	10 ± 0	9 ± 0	16 ± 0.57	7.3 ± 0.57
Aspergillus	-	-	9 ± 0	5 ± 0	7 ± 0	11 ± 0	6.16 ± 0.76	6.60 ± 0.57	10.66 ± 0.57	8 ± 0	6 ± 1	14.5 ± 1	-	-	-	18 ± 0.27	8.5 ± 0.27

Table 4: Effect of ethanolic extract of different developmental growth stages of pod and stem bark *C. fistula* on the growth of against *Fusarium, Aspergillus.*

Sr. No.	Name of the plant part	Name of organisms	
		Fusarium	*Aspergillus*
1	1 Month pod	10.33±0.57	9±0
2	4 Month pod	7.33±0.57	11±0
3	Pulp	8±0	10.66±057
4	Young stem bark	7±0	14.5±0
5	Old stem bark	-	-
6	Albendazole	16±057	18±0.27
7	Ethanol	7.3±0.57	8.5±0.27

Table No. 5: Minimum inhibition concentration of benzene extract of pod and stem bark of *C. fistula* against *Fusarium, Aspergillus.*

.	Diameter of zone of inhibition in mm														
	1month pod (C1)			4 month pod (C2)			Pulp (C3)			Young bark (C4)			Old bark (C5)		
Conc. in ml	**5**	**10**	**20**	**5**	**10**	**20**	**5**	**10**	**20**	**5**	**10**	**20**	**5**	**10**	**20**
Name of pathogen															
Fusarium	-	7±0	10±0	13.13±0.57	7.66±0.57	-	-	-	-	-	11±0	-	-	12±0	12±0
Aspergillus	7±0	6±0	8±0	6±0	-	-	-	-	-	-	-	9±0	-	-	-

Table 6: Effect of benzene extract of different developmental growth stages of pod and stem bark *C. fistula* on the growth of against *Fusarium, Aspergillus.*

Sr. No.	Name of the plant part	Name of the organisms	
		Fusarium	*Aspergillus*
1	1Monthpod	10±0	8±0
2	4 Month pod	-	6±0
3	Pulp	-	-
4	Young stem bark	11±0	-
5	Old stem bark	12±0	-
6	Albendazole	16±057	18±0.27
7	Benzene	-	-

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