Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2010, 1 (1): 166-172

Conservation of medicinal plant *Hibiscus rosa sinensis* through *Allium fistulosum* plantation

Rakesh Das

Department of Pharmacology, IPS College of Pharmacy, Gwalior, M.P., India

---

ABSTRACT

Hibiscus rosa- sinensis is a medicinal plant species which grows in moist soil favorable for the growth of Aspergillus niger (fungus) too. But extract of Hibiscus rosa- sinensis does n't shows inhibition zone against Aspergillus niger in PDA (Potato Dextrose Agar) media in microbiological evaluation. In other hand, the Allium fistulosum root extract having antifungal activity shows moderate zone of inhibition in PDA media. As the roots of the Allium fistulosum is much longer amongs the allium species it spreads networking formation inside the soil ground and changes the entire environment of soil, unsuitable for fungal growth. Thus, the plantation of Hibiscus rosa- sinensis as medicinal plant can be prevented from fungus Aspegillus niger, through cultivation of Allium Fistulosum within the rows of cultivated Hibiscus rosa-sinensis.

This antifungal medicinal plants not only aid in the conservation of Hibiscus rosa-sinensis but also it prevents the harvester's and garden workers life from Aspergillosis, a fatal respiratory fungal disease.

Keywords: *Hibiscus rosa- sinensis*, *Aspergillus niger*, Potato Dextrose Agar media, *Allium fistulosum* root extract, Aspergillosis**.**

INTRODUCTION

Hibiscus rosa-sinensis are native to Tropical Asia. A native of Southeastern Asia (China), the plant is commonly found through out the tropics and as a house plant through out the world. Most ornamental varieties are hybrids . *Hibiscus rosa sinensis* family Malvaceae is used as an Ayuvedic remedy for a long time described in Indian literatures and is mostly reported to having anti- diarrheic activities and antipyretics . The leaves are useful in healing of ulcers and promoting hair growth activity . The flowers of the plant possess

anti-spermatogenic, androgenic and anticonvulsant activity. The recent reports represents cardio protective, wound healing, anti- ammonemic, antioxidant, anti-tumor, post-coital anti-fertility and wound healing activities . Disordered of lipid metabolism following oxidative stress are the basic risk factors for initiation and progression of these diseases . Also recent investigation reports shows hypolipidemic activity of Hibiscus rosa sinensis root in triton-WR-1339 and high fat diet-induced hyperlipidemia in rats [1] .

A new unsaturated fatty acid monoglyceride, glycerol mono-(*E*)-8,11,12-trihydroxy-9-octadecenoate, was isolated from the seeds of *Allium fistulosum* L. along with five known compounds: tianshic acid, 4-(2-formyl-5-hydroxymethylpyrrol-1-yl) butyric acid, *p*-hydroxybenzoic acid, vanillic acid, and daucosterol [2].

A novel antifungal compound, fistulosin (octadecyl 3-hydroxyindole), was isolated from roots of Welsh onion (*Allium fistulosum* L.), and its structure was elucidated by spectroscopic means. This compound showed high activity against *Fusarium oxysporum* primarily inhibiting protein synthesis [3].

Root lengths of uninoculated plants ranged from 162.90 cm to 507.62 cm [4,5].

MATERIALS AND METHODS

2.1. Preparation of Root Extract

The roots of *Hibiscus rosa sinensis* was collected from the medicinal garden of IPS C ollege of pharmacy and was washed . Roots were dried under shade, powdered and (450 gm) extracted with 95% ethanol [1] in soxhlet extractor for 72 hrs, and extract was concentrated to driness under reduced pressure and controlled temperature (50-60 °C), yielding 20 gm of redish semisolids extracts. This was allowed to store in refrigerator .

2.2. Preparation of Microbial Growth of Aspergillus niger in Potato Dextrose Broth:

Potato infusion was made by boiling 300g of sliced (washed but unpeeled) potatoes in water for 30 minutes and then decanting or straining the broth through cheesecloth. Distilled water was added such that the total volume of the suspension is one litre. 20g dextrose and 15g agar agar powder was then added and the medium is sterilized by autoclaving at 15psi on 121°C for 15 minutes [6,7,8,9]

1.2.1. Potato Dextrose Broth composition -

Potato Starch ..... 4.0 g
Dextrose ..... 20.0 g
Agar ..... 15.0 g

2.2.2. Test Procedure: Pour Plate Methods [10,11,12,13]

1. 1 mL of test sample (*Aspergillus niger- MTCC-1344*) was added to a sterile petri dish.
2. The specified amount (10 or 20 mL) of sterile, molten agar (cooled to 45 - 50°C) was added and swirl gently to mix well.

The medium should be adjusted to a pH of approximately 3.5 with sterile 10% tartaric acid to inhibiting bacterial growth, which was gradually raised to 5.6 ± 0.2 at 25°C finally and was used

the standard pour plate technique. The plates were incubated at 25-30°C in an inverted position (agar side up) with increased humidity. Allow to solidify.

3. Incubation was performed at 22 - 25°C for 2 - 7 days or longer .

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25-30°C and a duplicate set at 35 ± 2°C. All cultures should be examined at least weekly for fungal growth and should be held for 4-6 weeks before being reported as negative.

4. Two cultured plates were made, one for the test of antifungal activity of root extract of *Hibiscus rosa-sinensis* and another for the antifungal activity of *Allium fistulosum*.

2.3. Antifungal activity evaluation of *Hibiscus rosa sinensis*

The developed culture of Aspergillus niger was inoculated with the 10% ethanolic extract of *Hibiscus rosa sinensis* in three different points to evaluate the zone of inhibition, marked A in disc shape microfilter paper. It was incubated for 2days at 30°C. The Figure 1 represents the remark.

2.4. Extraction of *Allium fistulosum*:

The roots for oil were dried and ground to semi-powdered state. The air-dried ground root parts (50 g) were hydro distilled in a clevenger apparatus (IPS College of Pharmacy) for 5 h. in accordance with the British pharmacopoeia. The yield was evoled 0.60% dry weight. The aqueous phase was extracted with dichloromethane (Qualigens) (3 x 50 mL). The organic phase was dried with sodium sulphate (CDH) , filtered and the solvent evaporated until dryness by air-dry. The fractions obtained were combined into calibrated flasks, evaporated to dryness and weighted in order to determine the extraction's efficiency. The oils were solublized in DMSO (LOBA CHEME) to a final concentration 5 mg/mL **[**14**].** The oils were stored in a sealed glass vial (bijoux bottle) in a refrigerator at 4 ºC until required. These all oils of above plants were screened for their antimycotic activity.

2.5. Antifungal activity evaluation of *Allium fistulosum* :

The methanolic extracts of plant extracts (*Allium fistulosum*) were screened for antifungal activity by agar well diffusion method (Perez et al., 1990) with sterile cork borer of size 6.0mm. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a petri dish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of methanolic extracts of *Allium fistulosum* plant extracts were introduced. Incubation period of 24-48hours at 28°C was maintained for observation of antifungal activity of plant extracts. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts. The antifungal analysis was carried out under strict aseptic conditions [15].

2.6. Plantation of *Hibiscus rosa sinensis* between *Allium fistulosum* rows:

The medicinal plants of *Hibiscus rosa sinensis* in medicinal garden planted in rows and between the rows the plantation of *Allium fistulosum* were also planted to evaluate the anti-fungal activity of *Allium fistulosum.*

RESULTS AND DISCUSSION

According to the figure 1 and table 1, the inoculation of *Hibiscus rosa sinensis* in *Aspergillus niger* culture of Potato Dextrose Agar (PDA) medium does not shows any antifungal activity represent by letter F shows no zone of inhibition i.e, 0 and average zone of inhibition of A,B,C,D & E is 1.58, 1.47, 1.58, 1.55,1.32 respectively. The Average zone of inhibition of *Allium fistulosum* is 1.5 cm.

Table 1: Comparative representation zone of inhibition of *Allium fistulosum* and *Hibiscus rosa sinensis* with respects to their mean diameters

Concentration is 20µg/ml	**Dimensions of Zone of inhibition**					
	***Allium fistulosum* (in cm)**					***Hibiscus rosa sinensis* (in cm)**
Samples	A	B	C	D	E	F
Diameters	1.5	1.5	1.7	1.7	1.3	0
	1.8	1.6	1.7	1.4	1.3	0
	1.62	1.4	1.5	1.6	1.41	0
	1.43	1.4	1.44	1.5	1.3	0
Average	1.58	1.47	1.58	1.55	1.32	0

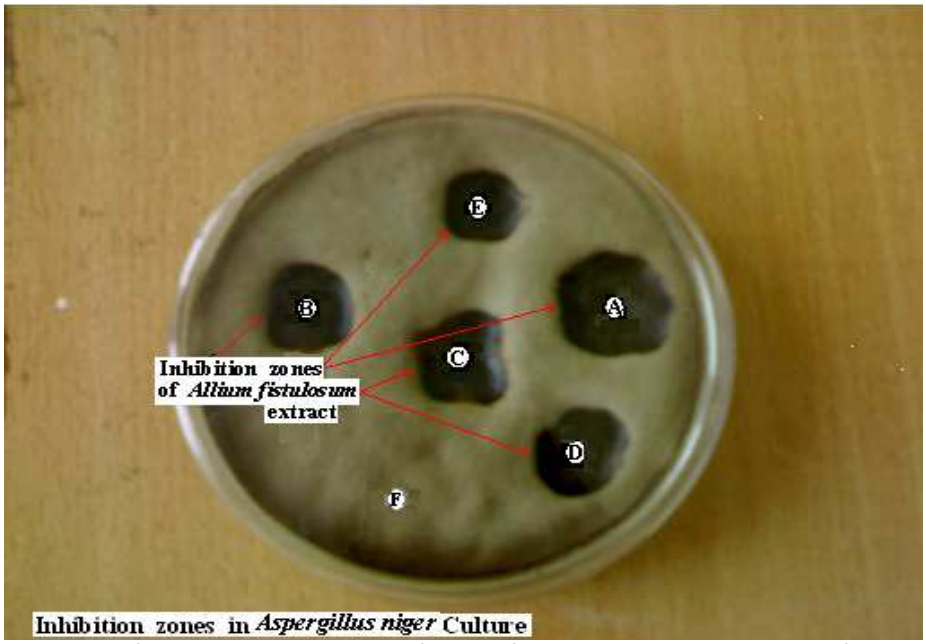
Table 2: Comparative study of zone of inhibition for Miconazole nitrate & *Allium fistulosum*

Samples	**Zone of inhibition Comparatively (in cm)**	
	Miconazole Nitrate	***Allium fistulosum***
Diameters	2.3	1.3
	2.2	1.5
Average	2.25	1.4

This means that *Hibiscus rosa sinensis* has the susceptibility to get fungal infection. As may be due to the climatic condition of *Hibiscus rosa sinensis* is good for the growth of *Aspergillus niger* i.e., moist climate and the root and stem has the dextrose as constituent.

In calculated values in table 3 represents average zone of inhibition of miconazole is 2.25 cm with compare to average zone of inhibition of *Allium fistulosum* is 1.4 cm in same petridish, PDA medium shown in figure 3. So, the antifungal activity of *Allium fistulosum* is low comparatively against Miconazole nitrate, but its works prominently . Thus expected antifungal activity of *Allium fistulosum* by resisting *aspergillus niger* are seen in moist soil of medicinal garden plantation of *Hibiscus rosa sinensis.*

Figure 1: Representation of the inhibition zone of *Allium fistulosum* against *Aspergillus niger*



A,B,C,D,E is the sample of 20 µg/ml concentration of *Allium fistulosum* and F is the sample of *Hibiscus rosa sinensis.*

Figure 2: Representation of the diameter of inhibition zone dimension (in cm) by *Allium fistulosum* in initial stage of mycelium growth *Aspergillus niger*

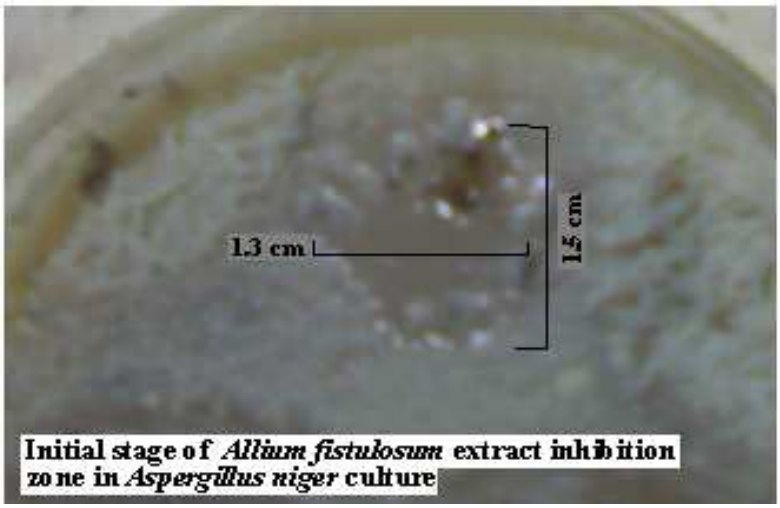
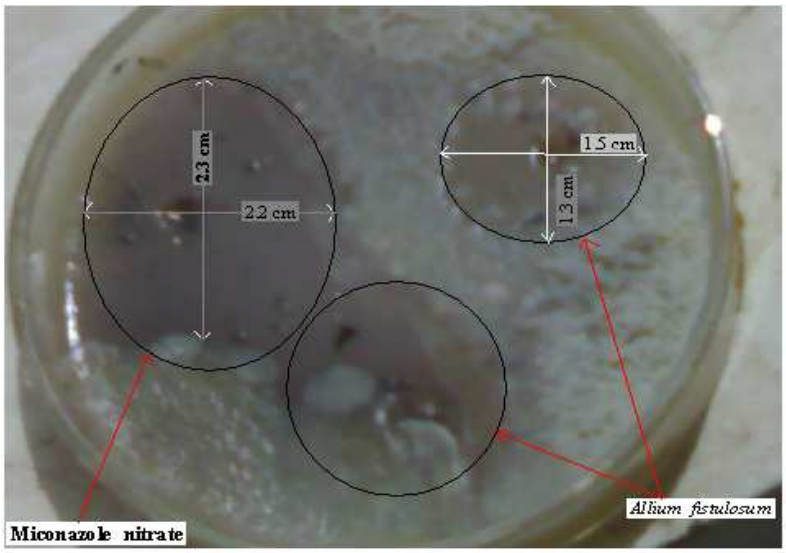


Figure 3: Representation of the zone of inhibition of miconazole nitrate (in cm) in comparable to *Allium fistulosum*



Plantation of *Hibiscus rosa sinensis* between *Allium fistulosum* rows shows resistant to fungal growth of *Aspergillus niger* to *Hibiscus rosa sinensis* even after spreading the mycelium . So, the plantation of *Allium fistulosum* which is acquiring long roots i.e, network forming environment of antifungal roots constituent surrounding the *Hibiscus rosa sinensis* root resist the growth of fungus *Aspergillus niger*.

CONCLUSION

The medicinal plant Hibiscus rosa sinensis is having many pharmacological activity, therapeutic activity but the fungal infection used to ruin the medicinal properties of this drug. But plantation of Allium fistulosum (itself has medicinal values) not only save the medicinal species but also save the life of harvester or garden care taker from a pulmonary disease Aspergillosis.

Acknowledgements
I woud like to thanks my parents , brother and my wife for inspiration during my research work. I am grateful for my institute IPS College of Pharmacy for providing extra time and materials.

REFERENCES

[1] V Kumar, P Singh, R Chander , F Mahdi, S Singh, R Singh, A K Khanna , JK Saxena , AA Mahdi , VK Singh and R K Singh, A Chatterjee & SC Prakashi. *Indian Journal of Biochemistry & Biophysics*, **2009**, 46, 507-510.
[2] S Sang, A Lao,Y Wang, CK Chin, RT. Rosen,and Chi-Tang Ho., *J. Agric. Food Chem.*, **2002**, 50 (22), 6318–6321.

[3] N Phay, T Higashiyama, M Tsuji, H Matsuura, Y Fukushi, A Yokota and F Tomita, *Phytochemistry*, **1999**., 52(2), 271-274 .
[4] K. Tawaraya, K. Tokairin and T. Wagatsuma. *Applied Soil Ecology*. **2001**, 17(2), 119-124.
[5] Melo, P.E. De. **2003**. The root systems of onion and *Allium fistulosum* in the context of organic farming: a breeding approach, 127.
[6] National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. Wayne, Pa: National Committee for Clinical Laboratory Standards; **1997**
[7] Gonzalez, G. M., Tijerina, R., Sutton, D. A., Graybill, J. R., Rinaldi, M. G., *Antimicrob. Agents Chemother*. **2002**, 46: 1583-1585
[8] J. H.Rex, P faller, M. A., Walsh, T. J., Chaturvedi, V., Espinel-Ingroff, A., Ghannoum, M. A., Gosey, L. L., Odds, F. C., Rinaldi, M. G., Sheehan, D. J., Warnock, D. W., *Clin. Microbiol. Rev.* , **2001**, 14: 643-658
[9] Pujol, I., Fernandez-Ballart, J., Guarro, J. *J Antimicrob Chemother*, **2001**, 47: 715-718
[10] Espinel-Ingroff, A. *Antimicrob. Agents Chemother*. **2001**, 45: 605-607.
[11] Yu Liu, G. Tortora, M. E. Ryan, HM Lee, and LM. Golub . *Antimicrob Agents Chemother*. **2001**, 46(5), 1455–1461.
[12] V Bobbarala, PK Katikala, K.C Naidu and S Penumajji. *Indian Journal of Science and Technology*, **2009**, 2(4), 87-90