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Role of isoflavones and its derivatives on the growth of *Aspergillus niger* and *Rhizactonia solani*

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Abstract
Two naturally occurring isoflavones genistine and biochanin - A, and their dihydro derivatives (isoflavones) as well as nine perhydroginated isoflavones (isoflavanes) were tested for their effects on mycelial growth of two soil fungi viz., *Aspergillus niger* and *Rhizactonia solani*. All the isoflavonenoids of the biochanin - A series showed the genistine isoflavane and the other isoflavanes with two hydroxyl groups and one methoxy groupe are fungi toxic, while isoflavones with two and one methoxy group were almost inactive.

Keywords: *Aspergillus niger*, isoflavones, mycelial growth, *Rhizactonia solani*

INTRODUCTION

The effects of isoflavonoids on microorganisms have been investigated by several groups of workers [1-2]**.** It has been established that some of them possess strong anti microbial activities [3-4]**.** However less information is available regarding the – structure activity relation ship of these compounds.

The evaluation of the flavonoids has to be understood in the light of their function in the environment in which they occur [5], the most important activities of isoflavonoids are dependent on the organisms in which they are present and is also related to isoflavonoid structure. As typical phenolic compounds, isoflvonoids act as potent antioxidants as conjugated aromatic compounds. They can act both as potent screens against destructive UV light and as well as attenuators of physiologically active visible light. But the most remarkable properties with regard to interferences with viral, bacterial, fungal and animal reproduction, growth and development which are revealed by few representatives of the flavonoids. These are especially isoflavones which, due to their structure simulate steroidal and other controllers of growth and development in there potential predators lignans which in their polymeric form can bind proteins, in changing enzymes, and other polymers such as polysaccharides and nucleic acids. These chemicals being heterocyclic phenols exhibit a close similarity in structure oestrogenic steroids.

The flavonoids in a number about four thousand, have closely related structures based on C-15 heterocyclic nucleolus of flavones varying chiefly in the number of phenolic methoxyl and other substituents. They are derived biosynthetically from the union of aromatic (hydroxylamile enzyme ester) and aliphatic (malonyl coenzyme A) precursors. About 500 million years ago evolution brought of biosynthesis. To the flavonoids flavonic apeginin, the basic flavonoid found along with sterols in the advanced blue green - alga (cyanobacteria), was product of dehydrogenation of the C-2, C-3 bond in flavones. The formation of flavones [6], in the blue green algae which inhibit the shores of lakes and streams, may have been a result of some mutation of systems which introduced double bonds in to the rings of steroids when about 420 years ago Plants invaded lands. They began to stiffen the internal and outer cell of there stems for upright growth and for further protection of the cell walls against potential enemies. During the course of lignifications, which involves polymerization of cinnamyl alcohols, lignans were formed, about 300 million years later, in the middle Cretaceous, the angiosperms arose and brought the essential changes such as exploitation of compounds for colour and pigmentation protection in flavonoid evaluation [6]. At this stage the isoflavones and their congeners formed a main class of flavonoid compounds affording antifungal anti bacterial and antiviral protections. These compounds make up the bulk of the phenolic antibiotic phytotoxins, compounds which are synthesized only when infection has started. Some are also potent insecticides and can act as estrogen mimics in mammals. Flavonoids secreted by plants may act as signals to initiate a co-operative activity symbiosis between soil bacteria belonging to the *Rhizobium* family and legumes [7] that leads to formation of nitrogen-fixing nodules in the legume root. Isoflavonoids can be isolated from most plant tissues [8], including leaves, stems, roots, flowers, seeds and germs. In germs and sprouts these compounds occur in abundance [9] and seem to regulate physiological processes important for plant growth.

MATERIALS AND METHOD

Screening for antifungal, antibacterial activity of synthetic and natural compounds

Natural as well as synthetic compounds are reported to have antifungal [10], antileukaemic [11], antibacterial [12], anticancer [13], anti-inflammatory 14], and gastroprotective activity [15]. Therefore, it is considered appropriate to screen the compounds obtained in the present study for their antifungal and antibacterial activity.

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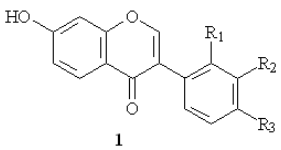
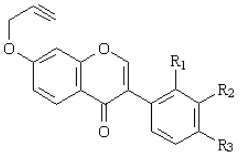


1

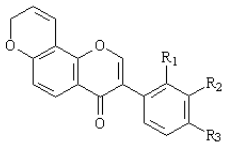
$R_1=R_3=Cl$, $R_2=H$



2 a-b

a) $R_1=R_3=Cl$, $R_2=H$

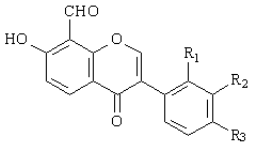
b) $R_1=Cl$, $R_2=R_3=H$



3 a-b

a) $R_1=R_2=R_3=H$

b) $R_1=R_3=Cl$, $R_2=H$



4 a-f

a) $R_1=R_2=H,R_3=OCH_3$

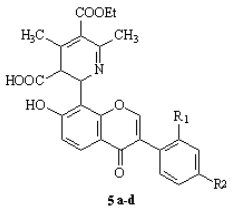
b) $R_1=R_3=OCH_3$, $R_2=H$

c) $R_1=R_2=H$, $R_3=Cl$

d) $R_1=Cl$, $R_2=R_3=H$

e) $R_1=R_2=H,R_3=Br$

f) $R_1=R_2=R_3=H$



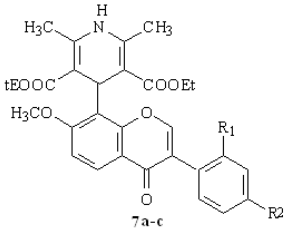
5 a-d

a) $R_1=R_2=OCH_3$

b) $R_1=R_2=H$

c) $R_1=R_2=Cl$

d) $R_1=Cl$, $R_2=H$

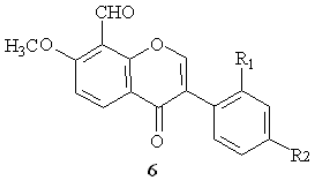


7 a-c

a) $R_1=R_2=H$

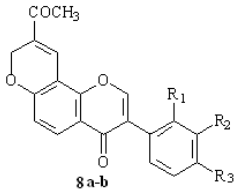
b) $R_1=R_2=Cl$

c) $R_1=Cl,R_2=H$



6

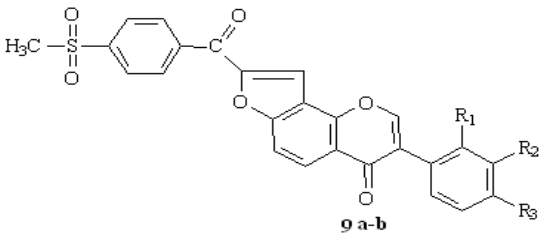
$R_1=R_2=H$



8 a-b

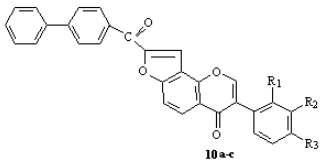
a) $R_1=R_3=H,R_2=Cl$

b) $R_1=R_2=H,R_3=Br$



9 a-b

a) $R_1=R_2=R_3=H$ b) $R_1=R_2=H,R_3=OCH_3$

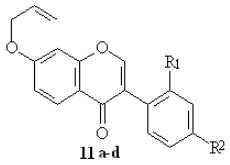


10a-c

a) $R_1=R_2=R_3=H$

b) $R_1=R_2=H,R_3=OCH_3$

c) $R_1=R_3=Cl,R_3=H$



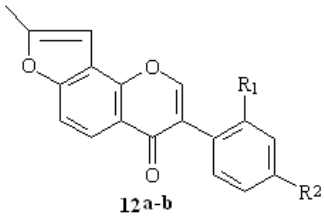
11 a-d

a) $R_1=R_2=H$

b) $R_1=H,R_2=OCH_3$,

c) $R_1=R_2=Cl$

d) $R_1=H,R_2=Br$



12 a-b

a) $R_1=H,R_2=Br$

b) $R_1=R_2=Cl$

The antifungal activity screening results of isoflavones, 7-propargyloxy-2',4'-dichloroisoflavone (2a), pyrano [2,3-f]-2',4'-dichloro-isoflavone (3b), 7-hydroxy-8-formylisoflavones (4b,c,e,f), 7-methoxy-8-formylisoflavone (6), 7-methoxy-8-[2'-(3",5"-dimethyl-4',6'-dicarbethoxypyridyl)]-2'-chloroisoflavone (7c), 8-methyl-2',4'-dichloro-4H-furo[2,3-h] isoflavone (12b). Section-B deals with antibacterial activity screening and results of isoflavones 7-hydroxy-2',4'-dichloro-isoflavone(1), 7-propargyloxy-2'-chloroisoflavone(2b), pyrano [2,3-f] isoflavone (3a),7-hydroxy-8-formylisoflavones (4a,c,e), 7-hydroxy 8-[2'-(4, 6- dimethyl- 3-carboxy- 5-cabe-thoxy- 2,3 dihydropyridyl)] isoflavone (5a-d),7-methoxy-8-[2'(3",5"-dimethyl-4',6'-dicarbe - thoxypyridyl)]isoflavones(7a-c),9-acetyl-pyrano[2,3-f]isoflavones (8a-b), 8-[4-methylsul -fonyl-benzoyl]-4H-furo[2,3-h]isoflavones (9a-b),8-[4-phenyl- benzoyl] -4H-furo [2,3-h] isoflavones (10a-c), 7-allyloxyisoflavones (11a-d), 8- methyl- 4'- bromo- 4H-furo [2,3-h] isoflavone (12a).

Antifungal activity

7-Propargyloxy-2',4'-dichloroisoflavone(2a),pyrano[2,3-f]-2',4'-dichloroisoflavone (3b), 7-hydroxy-8-formylisoflavones(4b,c,e, f),7-methoxy-8-formylisoflavone (6), 7-methoxy-8-[2'-(3",5"-dimethyl-4',6'-dicarbethoxypyridyl)]-2'-chloroisoflavone (7c), 8-methyl-2',4'-dichloro-4H-furo[2,3-h] isoflavone (12b) synthesized in the present study were tested for anti fungal activity. The evaluation of antifungal activity is carried out in the Department of Botany, Osmania University, Using paper disc method[16] and the fungal organisms used are *Aspergillus niger* and *Rhizoctonia solani* at concentration of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml.

Antifungal activity

The antifungal activity screening is done by the paper disc method [16].

Organisms used:i) *Aspergillus niger* ii) *Rhizoctonia solani*
Medium: Czapek-Dox Agar was used as culture broth.

Ingredients	g/1
Agar	15.0g
$NaNO_3$	2.0g
K_2HPO_4	1.0g
$MgSO_47H_2O$	0.5g
KCl	0.5g
$FeSO_47H_2O$	0.5g
Sucrose	30.0g
Distilled water	1 lit.

The pH of the medium, prepared from above ingredients is adjusted to 5.0-5.5. The medium was sterilized in the autoclave at 121 ^{0}C (15 lbs) pressure for 15 min. The medium was cooled to 45-50 ^{0}C and poured in 20ml volume in each petridish and allowed to solidify.

Testing equipments

Tubes of uniform size, paper discs and petridishes were employed.

Maintenance of sterility

All required apparatus were sterilized before use and necessary precautions were taken to avoid contamination.

Preparation of sample solutions

The testing sample 2 mg was dissolved in 2 ml of ethanol. This gives the concentration of the sample as 1000 µg/ml. Different dilutions such as 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and 6.25 µg/ml, were prepared from the sample solution.

Antifungal testing

After solidification of media, petriplates were inoculated with actively growing culture of *Aspergillus niger* and *Rhizoctonia solani* separately as follows. Filter paper discs of 5 mm diameter were dipped in the test solution of different concentration. After drying the disc, it was kept on czaeek-dox agar in petriplates seeded with *Aspergillus niger* and *Rhizoctonia solani* and incubated at 37 ^{0}C for 3-4 days.

RESULTS

The results are presented in Table-1. (+) Mark indicates inhibition of fungal growth (no growth), which indicates that the compound has antifungal activity, (±) mark indicates that there is a low growth in the culture, which indicates that the compound is less active, and (**–**) mark indicates the fungal growth, which means that the compound has no activity. The details of method of testing are given in experimental section.

Table 1. Antifungal activity

Comp.	*Aspergillus niger* (conc. µg/ml)						*Rhizoctonia solani* (conc. µg/ml)					
	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25
2a	±	±	±	–	±	–	±	±	–	–	±	–
3b	±	±	±	–	–	–	±	±	±	±	–	±
4b	+	+	+	+	+	+	+	+	+	+	+	+
4c	±	±	+	+	+	+	+	+	+	+	+	+
4e	+	±	±	±	+	–	–	+	+	+	+	+
4f	±	+	±	+	-	–	±	+	+	+	+	–
6	–	±	±	±	–	±	±	+	+	+	+	+
7c	±	+	±	–	–	–	±	+	±	–	–	–
12b	±	+	±	–	±	±	+	+	+	+	+	+

'+' indicates high activity '±' indicates less activity '-' indicates no activity

Among the compounds screened 7-hydroxy-8-formylisoflavones (4b,c,e,f), and 8-methyl-2',4'-dichloro-4H-furo[2,3-h]isoflavone (12b), have good antifungal activity at lower concentration towards both the strains *Aspergillus niger* and *Rhizoctonia solani.*

After 3-4 days the petridishes were checked for growth inhibition zone. The presence of clear zone of growth inhibition around the paper disc indicated the inhibition of growth of organism. The compound was considered to be active (+). If no clear zone or inhibition around the disc was observed in the petridish, it indicated no activity of the sample (-). If partial zone of inhibition was observed, it indicated the partial inhibition of growth (±). The antifungal activity of the compounds tested is given in table-1.

DISCUSSION

Among the compounds screened 7-hydroxy-8-formylisoflavones (4b,c,e,f), and 8-methyl-2',4'-dichloro-4H-furo[2,3-h]isoflavone (12b), have good antifungal activity at lower concentration towards both the strains *Aspergillus niger* and *Rhizoctonia solani.*

After 3-4 days the petridishes were checked for growth inhibition zone. The presence of clear zone of growth inhibition around the paper disc indicated the inhibition of growth of organism. The compound was considered to be active (+). If no clear zone or inhibition around the disc was observed in the petridish, it indicated no activity of the sample (-). If partial zone of inhibition was observed, it indicated the partial inhibition of growth (±). In the series of isoflavones are the most active substances inhibiting mycelia growth of *R. solani* 95% and *S. rolifisi* to the 91.6% at different concentrations. The isoflavones stimulated mycelia growth of *R. solani* at different concentrations. The isoflavones caused significant inhibition of mycelia growth only in the highest concentrations.

As in the case of 5,7,4' trihydroxy isoflavnes against *R.Solani* and *S.rolsii* in the present investigations it was found that the 7, 2, 4'-trihydrxy isoflavones ,dimethyl vestitol was fungi toxic to *Cladosparan cucumerinum* and *Aspergillus ni*ger . The 6, 7, 4' trihydroxy isoflavan was inactive against different fungi also. Also 6,

7, 4 trihydroxy isoflavanes/ isoflavones showed while the corresponding substances of the genistine series. They were active to some extant. Similar results were obtained by Adesanya *et al* [17] with genistine. The differences in activity between the 6, 7, 4' trihydroxy isoflavan/ isoflavanone and the substances may be ascribed to the presence of the hydroxyl groups at C-5. In the latter compounds. In the other investigations [16, 18, 19], it was found that even similer number of hydroxyl can be confer antifungal activity to isoflavones. This shows that the degree of reduction, the position and the number of hydroxyl group together constitute important parameters for high activity of the substances [20].

Among the tested substances a comparision of the effectiveness of the isoflavones. Genistine and Biochanine 'A' reveals that the presence of a methoxy group instead of hydroxyl group. Grow that C-4' bestows a high activity

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