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Role of isoflavones and its derivatives on the growth of *Pseudomonas putida and Escherichia coli*

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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 bacteria viz., *Pseudomonas putida and Escherichia coli.* All the isoflavonenoids of the biochanin - A series showed the genistine isoflavane and the other isoflavanes with two hydroxyl groups and one methoxy groups are toxic, while isoflavones with two and one methoxy group were almost inactive. Genistein, a radio protective soy isoflavone and protein kinase inhibitor, blocks the invasion of pathogenic bacteria in mammalian epithelial cells. The purpose of this study was to evaluate the direct effect of genistein on the survival and growth of the probiotic *Lactobacillus reuteri* and selected opportunistic bacteria *in vitro* as a prelude to *in vivo* use for managing post irradiation sepsis. The opportunistic bacterial enteropathogens viz., *Escherichia coli*, *Shigella sonnei*, and *Staphylococcus aureus* as well as *Klebsiella pneumoniae* and the non-pathogenic organism, *Bacillus anthracis* (Sterne) were evaluated. The latter two bacteria are found in the environment and may be of concern in irradiated individuals. A standard *in vitro* test was employed to evaluate the direct effect of genistein on the bacteria.

Keywords: *Escherichia coli*, isoflavones, mycelial growth, *Pseudomonas putida*

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, isoflavonoids 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.

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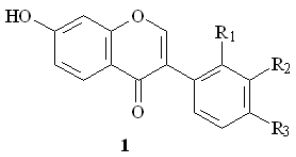
The flavonoids in a number about four thousand, have closely related structures based on C-15 heterocyclic nucleus of flavones varying chiefly in the number of phenolic methoxyl and other substituents. They are derived biosynthetically from the union of aromatic (hydroxylamile enzyme ester) and aliphatic (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 and seem to regulate physiological processes important for plant growth.

MATERIALS AND METHODS

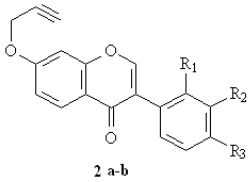
Screening for antibacterial activity of synthetic and natural compounds

Natural as well as synthetic compounds are reported to have antifungal [1], antileukaemic [9], antibacterial [10], anticancer [11], anti-inflammatory [12], and gastroprotective activity [5]. Therefore, it is considered appropriate to screen the compounds obtained in the present study for their antibacterial activity.



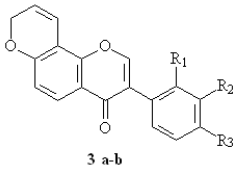
1

R_1=R_3=Cl, R_2=H



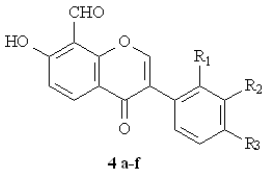
2 a-b

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



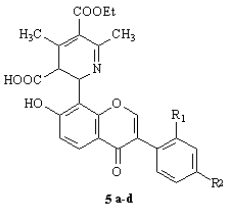
3 a-b

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



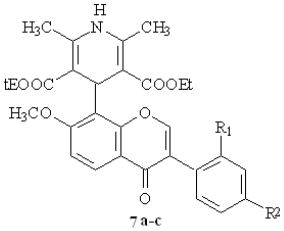
4 a-f

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



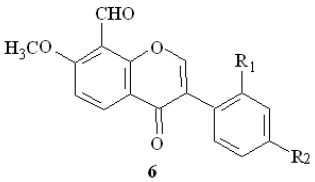
5 a-d

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



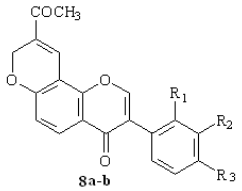
7 a-c

a) R_1=R_2= H
b) R_1=R_2= Cl
c) R_1= Cl,R_2=H



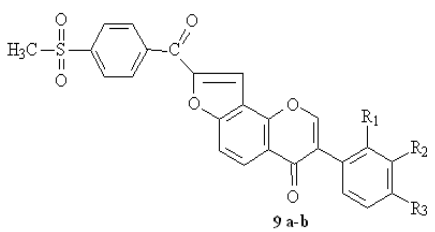
6

R_1=R_2=H



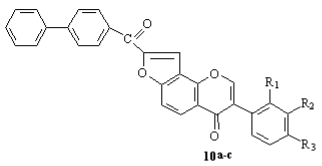
8a-b

a) R_1=R_3=H,R_2=Cl
b) R_1=R_2=H,R_3=Br



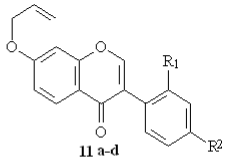
9 a-b

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



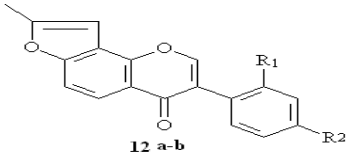
10a-c

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

Antibacterial activity:

7-Hydroxy-2',4'-dichloroisoflavone (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-cabethoxy-2,3-dihydropyridyl)]isoflavone(5a-d),7-methoxy-8- [2'-(3''5''-dimethyl-4',6'-dicarbethoxypyridyl)]isoflavones (7a-c), 9-acetyl-pyrano[2,3-f] isoflavones (8a-b), 8-[4-methylsulfonyl-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) obtained in the present study were evaluated for antibacterial activity. The evaluation of antibacterial activity is carried out in the Department of Botany, Osmania University, using paper disc method [11] and the bacterial strain used are *Pseudomonas putida* and *Escherichia coli* at 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml.

Antibacterial testing:

The antibacterial activity screening is done by the paper disc method [13].

Organisms used: *Escherichia coli* (Gram-positive bacteria) *Pseudomonas putida* (Gram-negative bacteria)

Medium: The antibiotic medium No.3 (Assay broth) was used as the culture broth.

Ingredients	g/l
Beef extract	1.5
Yeast extract	1.5
Peptone	4.0
Dextrose	1.0
Sodium chloride	3.5
Dipotassium phosphate	3.6
Monopotassium phosphate	1.2
Agar (1.5%)	15.0

The pH of the medium prepared from above ingredients adjusted to 7.0. 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 20 ml volume in each petridish and allowed to solidify.

Testing equipments

Tubes of uniform size, paper disc 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 DMSO. This gives the concentration of the sample as 1000 µg/ml. Different dilute solutions such as 200 µg/ml, 100µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml were prepared from the sample solution.

Antibacterial testing

After solidification of media, petriplates inoculated with actively growing culture of *Escherichia coli* and *Pseudomonas putida* separately as follows. Filter paper discs of 5 mm diameter were dipped in the test solution of different concentrations. After drying the disc, it was kept on Antibiotic med-3 agar in petriplates seeded with 1 ml bacterial culture of *Escherichia coli* and *Pseudomonas putida* and incubated for 24 hrs at 37 ^{0}C

RESULTS AND DISCUSSION

After solidification of media, petriplates inoculated with actively growing culture of *Escherichia coli* and *Pseudomonas putida* separately as follows. Filter paper discs of 5 mm diameter were dipped in the test solution of different concentrations. After drying the disc, it was kept on Antibiotic med-3 agar in petriplates seeded with 1 ml bacterial culture of *Escherichia coli* and *Pseudomonas putida* and incubated for 24 hrs at 37 ^{0}C

After 24 hours 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 inactiveness of the sample (-). If partial zone of inhibition was observed, it indicated the partial inhibition of growth (±). The antibacterial activity of the compounds tested is given in Table 1.

Table 1. Antibacterial activity

Comp.	*Pseudomonas putida* (conc. µg/ml)						*Escherichia coli* (conc. µg/ml)					
	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25
1	–	±	±	±	–	±	–	±	±	–	±	–
2b	–	–	–	–	–	–	+	+	+	+	+	±
3a	–	–	–	–	–	–	±	±	±	±	–	–
4a	–	–	–	–	–	–	±	±	±	–	±	–
4c	±	–	±	–	±	±	±	±	±	–	–	–
4e	–	–	–	–	–	–	±	±	±	±	±	–
5a	±	±	±	±	±	±	±	±	±	±	±	±
5b	–	±	±	±	–	±	–	–	–	–	–	–
5c	±	±	±	±	±	±	–	–	–	–	–	–
5d	±	±	±	±	±	±	±	±	±	–	±	±
7a	±	±	±	±	±	±	–	–	–	–	–	±
7b	-	±	±	±	-	±	+	+	+	+	+	±
7c	+	+	+	+	+	+	±	±	-	±	±	±
8a	±	±	±	±	±	±	+	+	+	+	+	+
8b	–	–	–	–	–	±	+	+	+	+	+	+
9a	±	±	±	±	±	–	±	±	±	±	±	±
9b	±	±	±	±	±	±	±	±	±	±	±	±
10a	+	+	+	+	+	+	±	±	±	±	±	±
10b	±	±	–	±	–	±	±	+	+	+	+	+
10c	±	±	±	±	±	±	+	+	+	+	+	+
11a	±	±	±	±	±	–	±	±	±	±	±	±
11b	–	–	–	–	–	–	+	+	+	+	+	–
11c	+	+	+	+	+	+	±	±	±	±	–	–
11d	–	–	–	–	–	–	±	±	±	±	–	–
12a	–	±	±	–	±	±	±	±	±	±	±	±

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

Genistein, a radioprotective soy isoflavone and protein kinase inhibitor, blocks the invasion of pathogenic bacteria in mammalian epithelial cells. The purpose of this study was to evaluate the direct effect of genistein on the survival and growth of the probiotic *Lactobacillus reuteri* and selected opportunistic bacteria in vitro as a prelude to in vivo use for managing post irradiation sepsis. The opportunistic bacterial enteropathogens *Escherichia coli*, *Shigella sonnei*, and *Staphylococcus aureus* as well as *Klebsiella pneumoniae* and the non-pathogenic organism, *Bacillus anthracis* (Sterne). The latter two bacteria are found in the environment and may be of concern in irradiated individuals. A standard in vitro test was employed to evaluate the direct effect of genistein on the bacteria. To screen six isoflavones isolated from *Erythrina poeppigiana* (Leguminosae) for their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Isoflavones from *E. poeppigiana* showed two different antibacterial activities against MRSA: direct growth inhibition and intensification of methicillin sensitivity.

Animals the latter showed definite healing properties. Significant antimicrobial action was detected in vitro and in vivo in phenothiazines that are applied to humans as neuroleptics or antihistamines. Both Gram-positive and Gram-negative bacteria

were equally sensitive, with the MIC varying between 25 and 100 µg/ml with most agents. Some phenothiazines were bactericidal, while others were bacteriostatic in action. Similar activity could be observed in isoflavononones obtained from the plants *Sophora* spp. Trifluoperazine and methdilazine exhibited antimycobacterial properties as well, and in experimental

Stem bark of *E. poeppigiana* was macerated with acetone and the methylene chloride-soluble fraction of the residue was applied to repeated silica gel column chromatography and eluted. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by a broth dilution method. Inactive compounds that failed inhibiting bacterial growth at 25 microg ml ($^{-1}$) were further investigated for their combination effects with methicillin and oxacillin. Of the isolated isoflavones, 5,7,4'-trihydroxy-8,3'-di (gamma,gamma-dimethylallyl)isoflavone (isolupalbigenin) exhibited the highest anti-MRSA activity (MICs: 1.56-3.13 microg ml(-1); MBCs: 6.25-12.5 microg ml(-1)), followed by 5,7,4'-trihydroxy-6-gamma,gamma-dimethylallylisoflavone (erythrinin B). Inactive compounds were combined with methicillin or oxacillin, 5,4'-dihydroxy-(3",4"-dihydro-3"-hydroxy)-2",2"-dimethylpyrano [5", 6":6,7] isoflavone (M-Wi-2) intensifying the susceptibility of MRSA strains to these antibiotics. In all but one strain, the MIC values of methicillin were reduced from > or =100 to 6.25-12.5 microg ml (-1) in the presence of M-Wi-2 (25 microg ml (-1)).Isoflavones from *E. poeppigiana* showed two different antibacterial activities against MRSA: direct growth inhibition and intensification of methicillin sensitivity [14].

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