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In Vitro Evaluation of Antibacterial Activity of Isoflavones

Nageswara Rao.G*, Ravi.G, Sharath Kumar Goud.S, Aparna.M, Javeria Uzma
Department of Pharmaceutical Chemistry,
Telangana University, Nizamabad,
Andhra Pradesh-503322

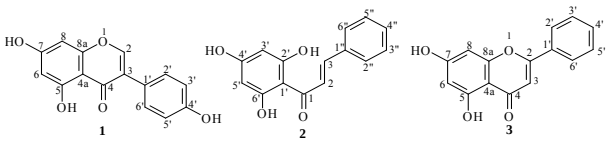
Abstract:
Natural as well as synthetic compounds are reported to have antifungal[1-3], antileukaemic[4], antibacterial[5], anticancer [6-8], antiinflammatory[9], and gastro protective activity[10]. Therefore, it is considered appropriate to screen the compounds obtained in the present study for their antibacterial activity. The anti-bacterial 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'-dicarbethoxy-pyridyl)] 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),**(+)-O-methylarmepavine **(13)**, paulownin **(14)**, diphyllin **(15)** and cleistanthin C **(16)**.

Keywords: Anti bacterial activity, Isoflavones, Natural compounds, Synthetic compounds.

1. INTRODUCTION

Plants and microorganisms elaborate a diverse range of heterocyclic compounds that are useful as drugs. The natural heterocycles are mainly of the classes of alkaloids, flavones, coumarins, chromones and isoflavones. Several synthetic analogs of these heterocyclics show different bioactivity. More than 50% of the drugs used in the modern medicine are based on either synthetic or natural heterocyclic systems.

A brief account of the biological activity oxygen heterocyclic compounds, which are used as drugs or in various stages of development as drugs.Isoflavonoids are large and very distinctive subclass of flavonoids. These compounds differ structurally from flavones in having the phenyl ring (ring B) attached to the 3 position of the heterocyclic ring. Genistein **(1),** a isoflavone, is derived biosynthetically by an aryl migration from the same chalcone **(2)** precursor that gives rise to apigenin **(3),** a flavone.Isoflavonoids are more restricted in the plant kingdom than other flavonoids since they are found regularly only in one subfamily of Leguminosae, the papilionoideae**.**



1 **2** **3**

2. MATERIAL AND METHODS:

Antibacterial testing:

The antibacterial activity screening is done by the paper disc method[11].

a) Organisms used:
   i) Escherichia coli (Gram-positive bacteria)
   ii) Pseudomonas putida (Gram-negative bacteria)

b) Medium:
   The antibiotic medium No.3 (Assay broth) and-Dox Agar was used as culture broth

Ingredients	*g/1*
Beef extract	1.5
Yeast extract	1.5
Peptone	4.0
Dextrose	1.0g
Sodium chloride	3.5g
Dipotassium phosphate	3.6g
Monopotassium phosphate	1.2g
Agar (1.5%)	15g.

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:

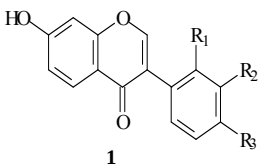
All required apparatus were sterilized before use and necessary precautions were taken to avoid contamination.

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.

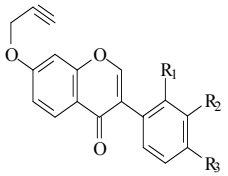
Antibacterial testing:

Antibacterial testing was done by the paper disc method[11]. 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.



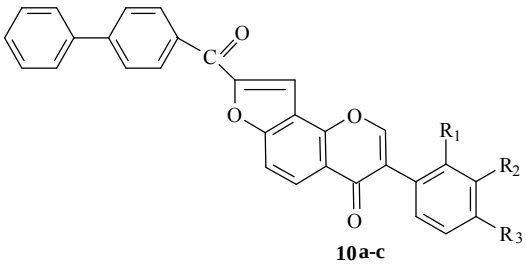
1

R_1=R_3=Cl , R_2=H



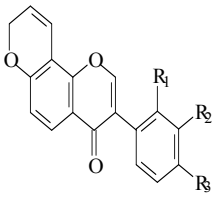
2a-b

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



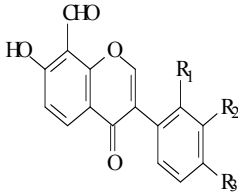
10a-c

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



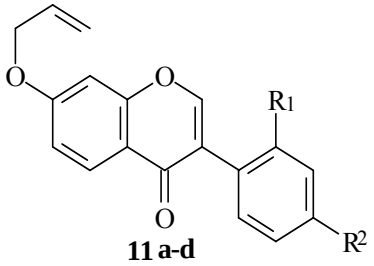
3a-b

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



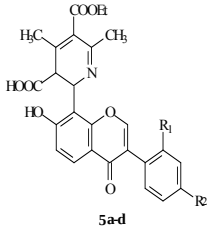
4a-f

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



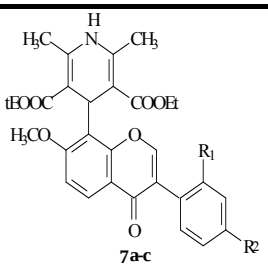
11a-d

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



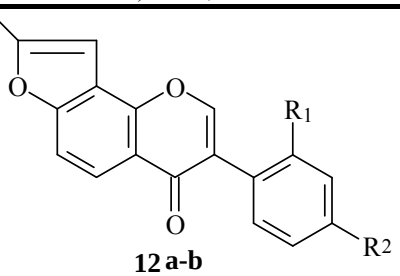
5a-d

a) R_1 = R_2 =OCH_3
b) R_1=R_2=H
c) R_1= R_2=Cl



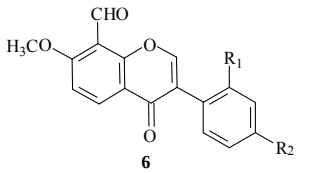
7a-c

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



12a-b

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



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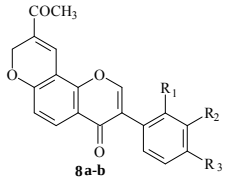
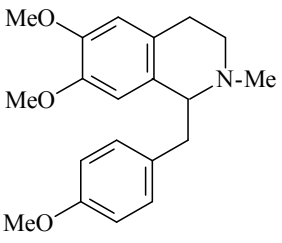


8a-b

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



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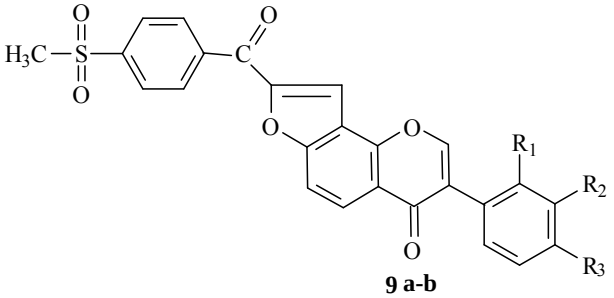
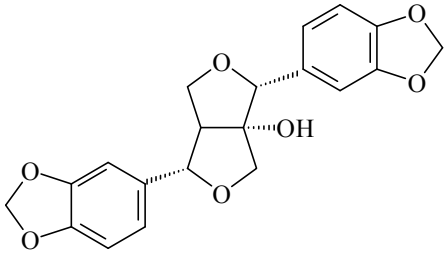


9a-b

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



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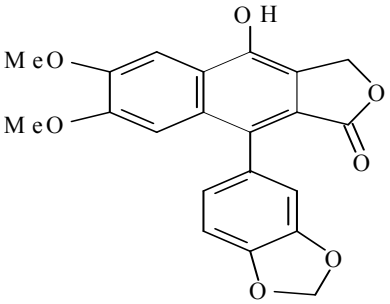

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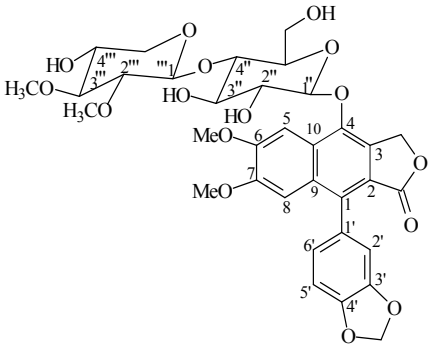

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Table 1 -Antibacterial activity

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	–	±	±	–	±	±	±	±	±	±	±	±
13	+	+	+	+	+	+	±	±	+	+	+	–
14	+	+	+	+	+	+	+	+	±	+	+	+
15	±	±	±	±	±	±	–	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	+	+

‘+’ indicates high activity **‘**±’ indicates less activity ‘-‘indicates no activity

Antibacterial activity:

7Hydroxy2’,4’dichloroisoflavone**(1),**7propargyloxy2’chlor oisoflavone**(2b),**pyrano[2,3f]isoflavone **(3a)**, 7-hydroxy-8-formylisoflavones **(4a,c,e),** 7-hydroxy8[2’(4,6 dimethyl3 carboxy5c abethoxy2,3dihydropyridyl)] isoflavone **(5ad)**, 7methoxy8[2’(3’’5’’dimethyl4’,6’dicarbethoxypyridyl)]iso flavones **(7a-c),** 9-acetyl-pyrano[2,3-f] isoflavones **(8a-b)**,8[4methylsulfonybenzoyl] 4Hfuro[2,3h] isoflavones**(9ab),**8 [4phenylbenzoyl]4Hfuro[2,3h] isoflavones**(10ac),**7allyloxyisoflavones **(11a-d)**, 8-methyl-4’-bromo-4H-furo[2,3-h]isoflavone**(12a),** (+)-O methylarmepavine **(13)**, paulownin **(14)**,diphyllin **(15)** and cleistanthin C **(16)**,obtained in the present study were evaluated for antibacterial activity.

The evaluation of antibacterial activity is carried out in the Department of Botany, Telangana 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. The results are presented in Table (+) mark indicates inhibition of bacterial growth (no growth), which indicates that the compound has bacterial activity, (±) mark indicates that there is a low growth in the culture, which indicates that the compound is less active, and (–) mark indicates the bacterial growth, which means that the compound has no activity. The details of method of testing are given in experimental section.

RESULTS AND DISCUSSION

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.

Among the natural compounds isolated from the plant of Cleistanthus collinus paulownin **(14),** cleistanthin C **(16)** showed a very good activity against both the strains Pseudomonas putida and Escherichia coli at different concentrations

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