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ANTI-BACTERIAL AND ANTI-HELMINTHIC ACTIVITY OF NOVEL HYDROXY FLAVONES

Pharmacology

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ABSTRACT

Antibacterial and anthelminthic effect of 7 hydroxy flavone and its derivatives were tested against human pathogens such as Enterococcus faecalis, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, and Aspergillus flavus using the Disc diffusion method. All the flavones showed significant activity against all pathogens. 7-THF showed a maximum zone of inhibition against all microorganisms and helminths tested. 7-hydroxy flavones showed the lowest antimicrobial and anthelminthic activity. 7 tri hydroxy flavone could be a possible source to obtain new and effective compounds to treat bacterial and helminthic infections.

KEYWORDS

Flavones, Gentamicin, Albendazole, Anti-Bacterial, Antihelminthic activity.

INTRODUCTION:

Increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide. In general, it is observed that pathogenic viruses, bacteria, fungi, and protozoa are more and more difficult to treat with the existing drugs [1]. To overcome the drawbacks of the current antimicrobial drugs and to obtain more efficacious drugs, an antimicrobial drug having a novel mode of action should be developed[2]. Plant-derived flavonoids are a large group of naturally occurring phenyl chromones found in fruits, vegetables, tea, and wine. They have been shown to have a wide range of biological activities, including antiallergic, antibacterial, antidiabetic, anti-inflammatory, antiviral, antiproliferative, antimutagenic, antithrombotic, anticarcinogenic, hepatoprotective, oestrogenic, insecticidal, and antioxidant activities[3].Additionally, some flavonoids are formed as antimicrobial barriers in plants' response to microbial infection. Therefore, it should not be surprising that they have been found in vitro to be effective antimicrobial compounds against a wide array of microorganisms. Recently, there has been an enormous increase in the number of studies on flavonoids as potential antimicrobial agents [4,5,6] in this study, antibacterial, and anthelminthic activities of three flavonoids (7hydroxy flavone, 7,3',4'tri hydroxy flavone, and 7,8,3'tri hydroxy flavone) were used.

MATERIALS AND METHODS:

Collection and Identification of flavonoids: These 7 hydroxy flavones and their derivatives were selected for the study and these were purchased from Sigma Aldrich, USA. The test compounds were prepared as fine powder. the flavons selected for the present study are 7hydroxy flavone, 7,3',4'tri hydroxy flavone, and 7,8,3'tri hydroxy flavone. This flavone is screened for anti-microbial activity.

Drugs and chemicals: Gentamicin (Salvavidas Pharmaceutical, Surat, Gujarat, India.) Albendazole (Cipla Mumbai, India).

Bacterial Strains: The microbial strains were obtained from the Sipra Labs limited Sanath Nagar, Hyderabad, Telangana, India. The studied bacterial strains were *Escherichia coli* ATCC25922*, salmonella typhi* ATCC 12225*, Enterococcus faecalis* ATCC 12022, and *Vibrio cholera ATCC*39315, *Staphylococcus aureus* ATCC25923.

Disc diffusion method : Antibacterial was carried out using the disc diffusion method[8].Petri plates were prepared with 20 ml of sterile MHA (Mueller- Hinton Agar) and PDA (PotatoDextrose Agar) (Hi-media, Mumbai). The test bacterial and fungal cultures (100 µlof suspension containing 108 CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. Three different concentrations of the extracts (1.25,2.5 and 5 mg/disc) were used for anti bacterial activity.

STATISTICAL ANALYSIS: The data were analyzed by using the one-way analysis of variance (ANOVA) with the Graph pad instant demo version and a p-value of < 0.05 was considered as statistically significant. The mean and the standard deviation were calculated for each parameter in each group. All the experiments were done in triplicates by three different observers to avoid observational bias and any sources of error.

RESULTS:

Antimicrobial activity:

Antibacterial effects of **7-HF, 7,3',4'THF,** and **7,8,3'THF** were examined using the Disc diffusion method against human pathogens such as *Escherichiacoli*ATCC25922*, salmonella typhi* ATCC 12225*, Enterococcus faecalis* ATCC 12022,and *Vibrio cholera* ATCC39315**.** *Staphylococcusaureus*ATCC25923, All the extracts showed significant activity against all pathogens.The activity was quantitatively assessed on the basis of the inhibition zone (Table.1). 7**,8,3'THF**and 7,3',4'THFshowed maximum antibacterial activity for all bacterial strains tested. In the presence of 7,**8,3'THF**, the maximum inhibition zone diameter at 5 mg/ml was obtained,that is 21mm in *Escherichia Coli and*18 mm in *Salmonella Typhi* (Figure-1). Similarly, the 7-3',4'showed a maximum inhibition zone with a diameter of 19 mm in *Escherichia Coli* .The 7 hydroxy flavone (18mm) showed restrained and minimum activity. More specifically, all bacterial strains showed higher susceptibility to 7,8,3'THF.

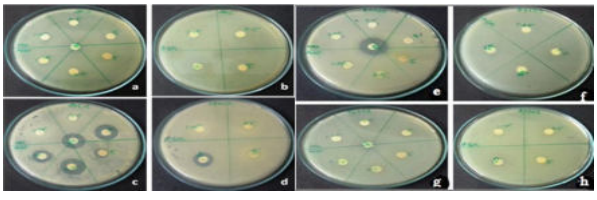

Figure 1 Antibacterial activity of 7-hydroxy flavone7,3',4' try hydroxy flavone7,8,3'try hydroxy flavone and 7,8,3'try hydroxy flavone

a&b - ***Staphylococcus aureus*ATCC25923;** c&d-Enterococcus faecalis (ATCC –12022)
e&f - ***Escherichia Coli*ATCC25922;** g&h- Escherichia coli (ATCC 25922)

Table 1

Name of the Pathogen	Antibacterial activity (Disc Diffusion Method) Zone of inhibition (mm) concentration of sample(mg)									
	7,8,3'THF			7,3',4' THF			7 -HF			Gentamycin (10 mg)
	1.25	2.50	5.0	1.25	2.50	5.0	1.25	2.50	5.0	
Escherichia Coli ATCC 25922	7	14	21	6	10	19	7	10	18	22
Salmonella Typhi ATCC 12225	6.2	8	18	7	9	17	6	9	14	21

Enterococcus faecalis ATCC 12022	5	10	17	6	8	16	6	8	13	24
*Vibrio cholera ATCC*39315	6.8	7.4	19	7	9	18	6	9	15	21
*Staphylococcus aureus*ATCC25923,	7.1	7.3	15	5	7	14	7.1	7.3	14	23

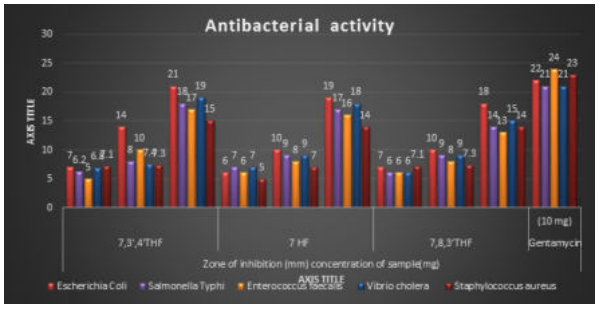

Figure 2

Anthelmintic Activity:

The use of the Ascaridiagalli species as a suitable model for the screening of anthelmintic drugs had been advocated earlier [9,10]. As the Indian earthworm, PheritimaPosthuma has an anatomical and physiological resemblance to the intestinal roundworm parasite of human beings, it was used for the initial evaluation of anthelmintic compounds in vitro [11,12]. The anthelmintic activities **of** 7hydroxy flavone, 7,3',4'tri hydroxy flavone, and 7,8,3'tri hydroxy flavone. against Pheretima posthuma at the concentrations of 50 and 100 mg/ml. The anthelmintic activities of three flavones were compared with Albendazole as the standard reference and with normal saline as the control. They were tested by a bioassay, which involved the determination of the time of paralysis (P) and time of death (D) of the worms. [13,14]. All the worms of the one species, Pheretima posthuma,were washed with normal saline to remove all the fecal matter and they were randomly selected for the anthelmintic study. The earthworms which were 4.5-8.5 cm in length and 0.1-0.3 cm in width were used for all the experimental protocols.

Experimental Design Six worms were released into 50 ml of the solutions (which were reconstituted with sterile water) of Albendazole (40mg/ml), the three flavones (50, and 100 mg/ mL), and normal saline (100mg/ml)[15]. The endpoint of the study was the time that was taken for the paralysis and/or the death of the worms. The worms are randomly divided into six groups (with six worms in each group).

Group – I Albendazole 50 mg/ml in 50ml solution
Group –II7,3',4' THF100 mg/ml in 50ml solution
Group – III 7-HF 100 mg/ml in 50ml solution
Group –IV 7,8,3'THF 100 mg/ml in 50ml solution
Group – V NS50 mg/ml in 50ml solution
Group -VI DW 50 mg/ml in 50ml solution

The time which was taken for the death of the worms was observed by two different persons and it was recorded after ascertaining that the worms neither moved when they were shaken vigorously nor when they were dipped in warm water (at approximately 500 C). The treatment with normal saline served as the control. The experiments were carried out in triplicates to avoid an observational bias and to minimize other sources of errors. Paralysis was said to occur when the worms do not receive even in normal saline. Death was said to occur when the worms lost their motility, followed by the fading away of their body color.

The 3 flavones, at the concentrations of 50mg/ml and 100mg/ml, produced an anthelmintic activity in a dose-dependent manner [52.94min (P-paralysis), 130.92min (D-death) and 50.42 min (P), 132.39 (D) respectively] [Table/Fig-2]. At the dose of 100 mg/ml concentration giving shortest time of paralysis (P) and death (D) which is significant with results of the control group and comparable with the Albendazole treatment group 42.84min (P) and 84.52 min(D).

Table:2

Group	Concentrations (mg/ml)	Time is taken for paralysis (min)	Time is taken for death of worms (min)
Group-I(Albendazole)	**50**	**42.84 ± 3.64**	**84.52 ± 7.45**
GroupII	**50**	**52.94 ± 3.2**	**130.92 ± 8.51**
(7,3',4'-THF)	**100**	**50.42 ± 1.24**	**132.39 ± 4.51**
Group -III	**50**	**53.91 ± 2.51**	**136.21 ±2.64**
(7,8,3'-THF)	**100**	**50.61 ± 1.35**	**132.35 ±4.51**
Group -IV	**50**	**55.31 ± 1.45**	**138.21 ± 3.26**
(7-HF)	**100**	**50.26 ± 2.31**	**134.21 ± 4.21**
Group -V (NS)	**0.9%**	**>180**	**>180**
Group -VI	**--**	**>180**	**>180**

***Anthelmintic activity 7,3',4'-THF of and its derivatives against Pheretimaposthuma**
+ SD, > More than, x - P < 0.001, NS- Not Significant.

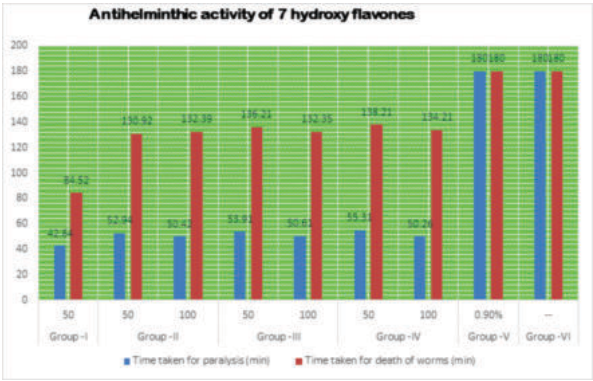

Figure 3 Antihelmintic activity of novel hydroxy flavones

DISCUSSION AND CONCLUSION:

Most of the anthelmintic drugs act by either killing or expelling the infesting helminths without harming the host. Albendazole, the congener of mebendazole, acts by increasing the chloride ion conductance of the worm muscle membrane, leading to hyperpolarization, thus causing a flaccid paralysis that results in the expulsion of the worm by peristalsis of the host gastrointestinal tract . The standard Albendazole solution showed enhanced anthelmintic activity. Among the tested novel flavones, 7,3',4'THF had significant antihelmintic activity.These flavonoids have multiple biological activities that may be responsible for their anthelmintic activity. In conclusion, the novel compounds of 7 hydroxy flavone,7,3',4'tri hydroxy flavone, and 7,8,3'try hydroxy flavone have been confirmed. Extensive in vivo research is needed to determine the individual components which are responsible for the anthelmintic activities of these flavonoids and the molecular mechanisms which are responsible for the same.

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