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Antimicrobial evaluation of some synthesized Flavone derivative compounds

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Abstract: The antibacterial evaluation of flavone derivative compounds was done against pathogenic micro-organisms. For the antibacterial activity against, four human pathogens namely, *Bacillus subtilis, Proteus vulgaris* and *Escherichia coli* and one plant pathogen, *Pseudomonas auroginosa* were selected. Antibacterial activity was tested by well method. Most of these compounds showed antibacterial activity against these selected pathogenic microorganisms. The MIC (Minimal Inhibition Concentration) of the flavones derivative was also tested against the above pathogens. The antifungal activity of flavones derivative were tested against four pathogenic fungi namely *Alternaria sp.* and *Helminthosporium sp.*, *Aspergilus niger* and *A. flavus*. The antifungal activity was tested by well method. Most of these compounds showed antifungal activity.

Keywords: Flavones, Antibacterial, Antifungal

INTRODUCTION

Flavones are commonly found in human diet especially in fruits, vegetables, tea, red wine, and juices. Consumers and food manufacturers are interested in flavones because these compounds could exert direct or indirect beneficial effects on health [1, 2]. The flavone backbone derives from a chalcone intermediate and consists of two aromatic rings interconnected by a three carbon atom heterocyclic ring. The subsequent modifications on this polyphenolic structure can lead to the great diversity of flavone derivatives. Among their numerous biological activities including antioxidant, antitumor, and anti-inflammatory properties, some flavone derivatives were shown to be active on chloroquine-sensitive and resistant strains of *P. falciparum* [3, 4, 5]. Major flavanols found in human diet, such as apigenin, luteolin and quercetin, can inhibit parasite growth by targeting metabolic pathways occurring in the apicoplast a plastid-like organelle encountered in apicomplexan parasites. Besides a direct inhibition of the parasitic metabolic pathways, flavones could disturb the infected-erythrocyte cytoadherence properties and decrease their sequestration in small vessels. Several flavones have an interesting anti-HIV activity [6]. The objectives of the study were to determine antimicrobial activity of flavones derivatives and to determine the MIC (Minimal Inhibition Concentration) of the flavones.

MATERIALS AND METHODS

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Collection of chemical compounds:-

All the six synthesized compounds were collected from the department of chemistry, Yeshwant Mahavidyalaya, Nanded.

Culture

The synthesized compounds were subjected for antibacterial activity against four bacterial strains viz., *Proteus vulgaris, E.coli, Bacillus subtilis, Pseudomonas auroginosa* and four fungal species as *Helminthosporium sp. Alternaria spp. Aspergillus niger* and *Aspergillus flavus.* The pure culture of test bacteria were obtained from culture collection deposited in department and the pure culture of bacteria was maintained on selective sterile agar slants. The cultures were maintained in refrigerator for use and regularly checked for contamination, periodic transfer were made aseptically.

Preparation of solution of compound

The flavone derivative compounds were dissolved in the organic solvent, Dimethyl sulphoxide [DMSO] and dilution is made as in three concentrations of compounds as 50 µg, 100 µg and 250 µg compound in 1ml DMSO. The solution was then used for antimicrobial testing, MIC.

Determination of Antimicrobial activity of flavones derivatives

The antimicrobial study was conducted for the determination of following parameters like, Zone of Inhibition and MIC (minimum inhibitory concentration), Different concentration of synthetic chemical compounds were tested for anti-microbial activity by disc diffusion method [7]. Nutrient agar medium was inoculated with different micro-organisms and once the media was solidified, it was punched with a 6 mm diameter well [8]. The wells were then filled with different concentration of synthetic

chemical compounds and control (DMSO) (concentration of synthetic chemical compounds was 50 µg/ml ,100 µg/ml and 250 µg/ml). Agar plates containing bacteria and synthetic chemical compounds were incubated at 37ºC for 24 hrs. Antimicrobial activity was evaluated by measuring the inhibition zone. Inhibition zones were recorded as the diameter of growth free zone, including the diameter of the well, in millimeters at the end of the incubation period. The tested drug was classified as active when the diameter of the inhibition zone was equal to or larger than 6 mm. Simultaneously standard antibiotic Penicillin for P. *vulgaris*, *P. aeruginosa*, *E. coli*, *B. subtilis* were used for comparison at a conc. 50 µg/ml each. The sample was tested in triplicate. Similar procedure was adopted for fungi, except PDA was used as a selective media for the effective growth of fungi. Nystatin was used as standard antibiotic for the comparison of zone of inhibition with different concentrations of synthetic chemical compounds. At the end of incubation period the zone of inhibition for the synthetic chemical compounds was measured for each bacteria and fungi and the results were tabulated.

The results for the comparison of MIC of synthetic chemical compounds with standard antibiotics were recorded. Synthetic chemical compounds in different concentrations (100 µg/ml, 250 µg/ml) control (DMSO) and standard (Nystatin 5 µg/ml), were transferred to the cups of each agar plate, incubated at room temperature (27^0C) and examined for inhibition zones after 36 hours of incubation to screen for antifungal activity. Microbial cultures and growth conditions

The synthetic chemical compounds were assayed for antifungal activity against the fungal strains, *A. niger*, *A. flavus*, *Heliminthosporium spp. ,Alternaria spp.*

RESULTS

Table 01. showing 50 µg compound dissolved in 1ml of DMSO

Sr. no	Comp.	Bacteria (Zone of inhibitions)							
		P. valgaris		*B. subtilis*		*E. coli*		*P. aerogenosa*	
		STD (Penicillin)	COMP.	STD (Penicillin)	COMP.	STD (Penicillin)	COMP.	STD (Penicillin)	COMP.
01	C_1	3mm	2mm	7mm	4mm	3mm	3mm	3mm	3mm
02	C_2	3mm	-----	8mm	4mm	3mm	-----	3mm	-----
03	C_3	3mm	-----	8mm	5mm	3mm	-----	3mm	2mm
04	C_4	3mm	5mm	7mm	3mm	3mm	4mm	3mm	4mm
05	C_5	3mm	6mm	7mm	4mm	3mm	7mm	3mm	4mm
06	C_8	3mm	5mm	7mm	3mm	3mm	-----	3mm	-----

In the present investigation, six flavone derivatives were selected as probable test chemicals and screened *in vitro* for their antibacterial and antifungal activities against eleven human pathogenic bacteria and four phytopathogenic fungi. It was observed that all the four bacteria such that *E.coli*, *Pseudomonas aerogeinosa*, *Proteus vulgaris*, and *Bacillus subtilis* shows antibacterial activity against chemical compounds with zone of inhibition.

When pathogenic bacterial strains were tested against flavones derivative compounds such as

C 1, C 2, C 3, C 4, C 5 & C 8 at different concentration (50 µg/ml, 100 µg/ml, 250 µg/ml) Some

compounds showed good inhibition area at one specific concentration but at varied concentration the above bacteria did not show or less area of zone of inhibition as compared to previous one. Some bacteria showed resistance to some given flavone derivative compounds at all concentrations so did not show any zone of inhibition.

Table 02. showing performance of 250 µg compound dissolved in 1ml of DMSO.

Sr. no	Comp.	Bacteria (Zone of inhibitions)							
		P. valgaris		*B. subtilis*		*E. coli*		*P. aerogenosa*	
		STD (Penicillin)	COMP.	STD (Penicillin)	COMP.	STD (Penicillin)	COMP.	STD (Penicillin)	COMP.
01	C_1	2mm	2mm	11mm	8mm	------	3mm	5mm	2mm
02	C_2	2mm	-----	14mm	6mm	------	-----	4mm	3mm
03	C_3	2mm	2mm	10mm	6mm	------	-----	6mm	4mm
04	C_4	2mm	5mm	10mm	6mm	------	5mm	4mm	5mm
05	C_5	2mm	5mm	10mm	5mm	------	4mm	4mm	5mm
06	C_8	2mm	-----	10mm	4mm	------	-----	5mm	5mm

The antifungal activity of flavone derivative compounds was tested against the four fungal species. *A. niger*, *A. flavus*, *Alternaria spp*. and *Helminthosporium spp*. at the two concentration levels i.e. at 100 µg/ml and 250 µg/ml. At 100 µg/ml, *A.niger* and *A. flavus* showed good zone of inhibition to all six flavone derivative compounds, but *Alternaria spp*. Did not show any zone of inhibition and the *Helminthosporium spp*. did not show zone of inhibition to the compound namely C2, C3 and C4 and at the concentration of 250µg/ml(as shown in table no.5). *Aspergillus niger* showed good zone of inhibition except for the compound C4 and *A. flavus* to C2, C4, C5 and C6. At this concentration, Alternaria showed good zone of inhibition to all six compounds and *Helminthosporium spp*. Showed zone of inhibitions to the compounds C3 and C5 only.

Table 03. showing performance of 100 µg compound dissolved in 1ml of DMSO.

Sr. sr.no	Comp.	Fungi (Zone of inhibitions in mm)							
		A. niger		*A. flavous*		*Alternaria sp.*		*Helminthosprium sp.*	
		STD (Nystatin)	COMP.	STD (Nystatin)	COMP.	STD (Nystatin)	COMP.	STD (Nystatin)	COMP.
01	C_1	3mm	5mm	-----	2mm	-----	-----	-----	-----
02	C_2	3mm	2mm	-----	2mm	-----	-----	-----	-----
03	C_3	3mm	3mm	-----	1mm	-----	-----	-----	-----
04	C_4	3mm	3mm	-----	2mm	-----	-----	-----	-----
05	C_5	3mm	4mm	-----	4mm	-----	-----	-----	4mm
06	C_8	3mm	2mm	-----	2mm	-----	-----	-----	2mm

Table 04 showing performance of 250 µg compound dissolved in 1ml of DMSO

Sr. no	Comp.	Fungi (Zone of inhibitions in mm)							
		A. niger		*A. flavus*		*Alternaria sp.*		*Helminthosprium sp.*	
		STD (Nystatin)	COMP.	STD (Nystatin)	COMP.	STD (Nystatin)	COMP.	STD (Nystatin)	COMP.
01	C_1	4mm	7mm	-----	5mm	4mm	6mm	2mm	-----
02	C_2	4mm	2mm	-----	----	4mm	4mm	2mm	-----
03	C_3	4mm	6mm	-----	3mm	4mm	6mm	2mm	3mm
04	C_4	4mm	-----	-----	-----	4mm	4mm	2mm	-----
05	C_5	4mm	4mm	-----	-----	4mm	6mm	2mm	3mm
06	C_8	4mm	3mm	-----	-----	4mm	3mm	2mm	-----

DISCUSSION

Our results suggest that against these compounds show significant antibacterial activity against test bacteria. Most of flavone derivatives show maximum zone of inhibition against gram negative bacteria as *E. coli*, *Proteus vulgaris*, *Psudomonas sp*. as well as gram positive as *Bacillus subtilis*. All the flavone compounds tested against four fungi as *Aspergillus niger*, *A. flavus*, *Alternaria sp*. *Heliminthosporium sp* showed interesting results.

Only two compounds, i.e. C_2 and C_4 did not show any zone of inhibition. These derivative compounds are could be used as the antibacterial and the antifungal agents in the pharmaceutical industries.

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