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Study on antibacterial activity of 7-[2-hydroxy-3(substituted amino)propoxy]-5-hydroxy-2-phenyl-4*H*-chromen-4-one

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ABSTRACT

Polyphenolic compounds have recently attracted considerable interest in the field of nutrition, health and medicine. This is the result of the growing body of evidence suggesting that these compounds may act as potent biological activities. Synthesized chrysin derivatives (FC1-FC8) were analysed for their antibacterial activity by using Kirby-Bauer disk-diffusion method on Mueller-Hinton agar according to the guidelines of the Clinical Laboratory Standards Institute, 2007, USA. All the synthesized compounds (FC1-FC8) were tested for their antibacterial activity *in vitro* with two gram-positive *Staphylococcus aureus* MTCC3160 and *Bacillus cereus* MTCC 430 two gram-negative bacteria *E.Coli* MTCC 442 and *Vibrio cholera* MTCC 3904. Amikacin and Gentamicin were used as standard control agents. Among all the tested flavonoids derivatives many compounds exhibited varying levels of antibacterial activity against all the Gram-positive as well as Gram-negative bacteria. It has also been observed that Gram-positive bacteria were more susceptible towards the newly synthesized series of compounds (FC1-FC8 series) as compared to Gram-negative bacteria.

Key words: Flavonoid, antibacterial activity.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds ubiquitous in many plants, in which they occur as the free forms, glycosides, as well as methylated derivatives. In general, flavonoids possess a chromane ring attached to an additional aromatic ring, which derives from malonyl-CoA and p-coumaroyl-CoA (Dixon et al. 1983). Flavonoids have been in focus due to their nutraceutical and therapeutical significance, as they exhibit divergent biological activities such as antioxidant, anti-inflammatory, cardioprotective, antibacterial, antitumor, hepatoprotective, antiviral activities (Kelly et al. 2002; Harborne et al. 1999; Kumar et al. 2013; Kumar et al. 2013; Leopoldini et al. 2006; Kumar et al. 2013; Cook et al. 1996).

On the other hand, microbial resistance has become a major concern all over the world and, therefore, ample investigation and research in this scope are dreadfully compulsory. Coming to the fore more recently is that there is an increasing interest in flavonoids due to their anti-infective properties (Rice-Evas et al. 1995). For instance, the flavonoids quercetin, kaempferol as well as the flavonoid glycosides rutin and isoquercitin were reported to have antibacterial and antifungal activities (Pandey 2007).

Recently, there has been an enormous increase in the number of studies on flavonoids as potential antimicrobial agents(Kallikat et al. 2009, Burkhart et al. 1997).

Here, we have reported antibacterial activity of synthesized flavonoid (chrysin fig. 1) derivatives (FC1- FC8). These compounds can be

used as chemotherapeutic agents for the treatment of diseases caused by different microorganisms.

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes or prevent the growth of

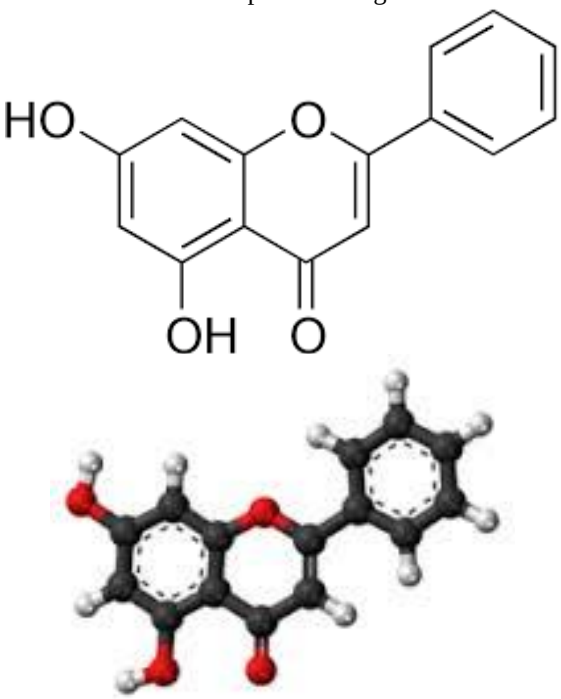


Fig. 1: Chrysin

microbes. Disinfectants are antimicrobial substances used on non-living objects.

Antibiotic resistance is a serious concern world wide as it would result in strains against which currently available antibacterial agents will be ineffective. In general, bacterial pathogens may be classified as either Gram-positive or Gram-negative pathogen. Antibiotics compounds with effective activity against both Gram-positive and Gram-negative pathogens are generally regarded as having a broad spectrum of activity. The synthesized compounds were preliminary screened Gram positive and Gram negative pathogens.

Bacteria cause serious infections in humans as well as other animals. For example, it was found that staphylococcus aureus causes superficial skin lesion and food poisoning (Wedel et al. 2001). Pseudomonas aeruginosa is a nosocomial pathogen accounting for a significant percentage of hospital- acquired infections and health care centers because there are a little effective antimicrobial agents against it (Babu et al. 2006).

The chemical structures of tested flavonoid derivatives shown in table 1. All the derivatives were derived from their parent compound.

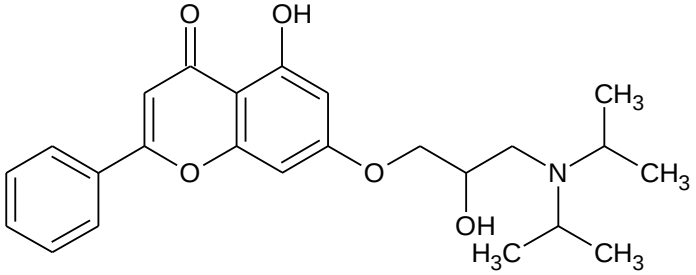
Aim

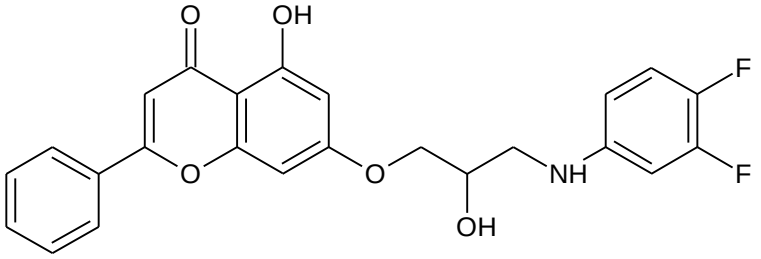
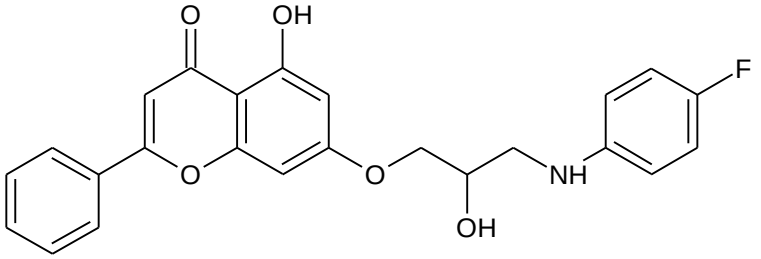
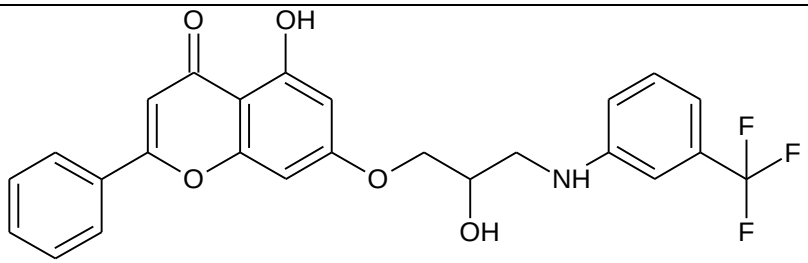
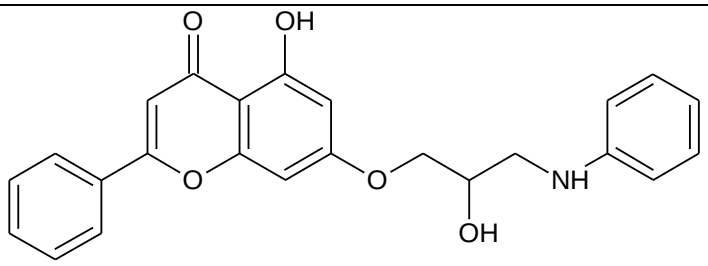
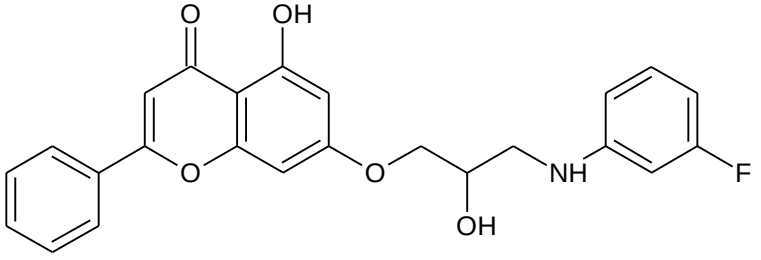
Infectious as well as highly contagious microbial diseases are increasing with course of time round the world due to the emergence of new multidrug resistant bacteria which are resistant to a number of antimicrobial agents due to the development of mutagenicity (Chan et al. 2000). One way to battle with this challenge is the conscious usage of the currently marketed antibiotics and the other is to develop and screen new chemical entities for antimicrobial activities (Lee et al. 1999).

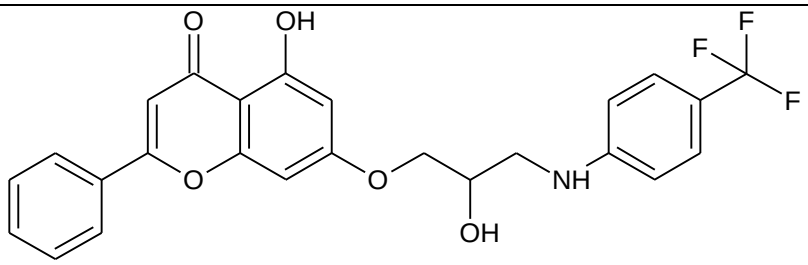
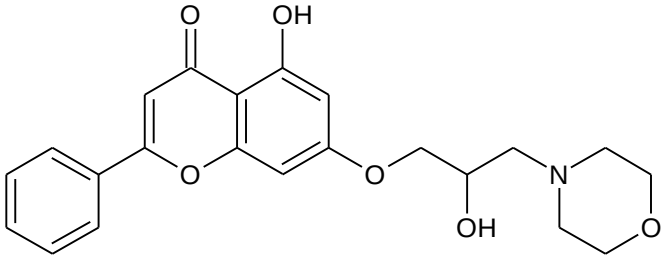
In view of this, it is imperative to discover new chemotherapeutic agents to prevent the emergence of resistance and ideally shorten the duration of therapy.

The synthesized compounds were analysed for their antibacterial activity by using Kirby-Bauer disk-diffusion method on Mueller-Hinton agar according to the guidelines of the Clinical Laboratory Standards Institute, 2007, USA (Shin et al. 1999).

Table 1: Structures of chrysin derivatives.

FC1	$C_{24}H_{29}NO_5$	 7-{3-[di(propan-2-yl)amino]-2-hydroxypropoxy}5-hydroxy-2-phenyl-4*H*-chromen-4-one

FC2	$C_{24}H_{19}F_2NO_5$	 7-{3-[(3,4-difluorophenyl)amino]-2-hydroxypropoxy}-5-hydroxy-2-phenyl-4*H*-chromen-4-one
FC3	$C_{24}H_{20}FNO_5$	 7-{3-[(4-fluorophenyl)amino]-2-hydroxypropoxy}-5-hydroxy-2-phenyl-4*H*-chromen-4-one
FC4	$C_{25}H_{20}F_3NO_5$	 7-(2-hydroxy-3-{[3-(trifluoromethyl)phenyl]amino} propoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one
FC5	$C_{24}H_{21}NO_5$	 7-[2-hydroxy-3-(phenylamino)propoxy]-5-hydroxy-2-phenyl-4*H*-chromen-4-one
FC6	$C_{24}H_{20}FNO_5$	 7-{3-[(3-fluorophenyl)amino]-2-hydroxypropoxy}-5-hydroxy-2-phenyl-4*H*-chromen-4-one

FC7	$C_{25}H_{20}F_3NO_5$	 7-(2-hydroxy-3-{[4-(trifluoromethyl)phenyl]amino} propoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one
FC8	$C_{22}H_{23}NO_6$	 7-[2-hydroxy-3-(morpholin-4-yl)propoxy]-5-hydroxy-2-phenyl-4*H*-chromen-4-one

MATERIALS AND METHODS

Kirby-Bauer disk-diffusion method (Cardenas et al. 2006) is a method to determine the sensitivity of microorganisms to specific antimicrobial drugs, greater drug efficacy yields larger microbe-free zones surrounding drug-containg disks after overnight growth on solid media.

Zone of inhibition – this is an area of media where bacteria are unable to grow due to presence of a drug that impedes their growth. The size of a zone of inhibition in a KB test is inversely related to the minimum inhibitory concentration (MIC), which is the amount of antibiotic required to prevent bacterial growth in an overnight culture.

Therefore, in this study, we aimed to examine *in vitro* antibacterial activities of flavonoid derivatives.

EXPERIMENTAL

All the synthesized compounds (FC1-FC8) were tested for their antibacterial activity *in vitro* with two gram-positive *Staphylococcus aureus* MTCC3160 and *Bacillus cereus* MTCC 430 two gram-negative bacteria *E.Coli* MTCC 442 and *Vibrio cholera* MTCC 3904. Amikacin and Gentamicin were used as standard control agents. The antibacterial activity (zone of growth inhibition) of FC1-FC9 series of compound were measured by Kirby-Bauer disk-diffusion method on Mueller Hinton agar according to the guideline of Clinical Laboratory Standards Institute, 2007, USA (CLSI. 2007)

We prepared the Mueller-Hinton agar according to the manufacture's instructions. Used media was sterilized at 121 °C for 20-25 min then cooled upto 50 °C. The 90 mm sterile petri plates filled with media. It was then kept for 15-20 min at room temperature for solidification. Plates were inverted and left for overnight in incubator to check the sterility of the plates. Overnight grown cultures of *S. aureus* MTCC 3160, *B. cereus* MTCC 430, *E. Coli* MTCC 442, and *V. cholera* MTCC 3904 were spreaded over the agar surface of the Mueller-Hinton agar plates. Whatman no. 1, filter paper disk impregnated with different synthesized compounds (0.5 mg/mL in DMSO) gently placed on the surface of plates using sterile forceps. The plates were inverted and incubated at 37 °C for 20-24 h. After some time we found clear zone of growth inhibition surrounding the disk. It was measured using antibiotic zone measurement scale and tabulated. All the tests were performed in twice and average was taken as the final reading. A large zone of inhibition around an antibiotic containing disk indicates that the bacteria are more sensitive to the antibiotic in the disk.

RESULTS AND DISCUSSION

From the data table, it is clear that the substitution in aryl ring exerted significant influence on the antibacterial activity of the synthesized flavonoid derivatives. The compound (substituted by amine) FC3 was found to be more active than the other compound of the series. This compound (FC3) showed better activity profile (zone of growth inhibition 63.98 mm) against *S. aureus* MTCC 3160, *B. cereus* MTCC 430, *E.coli* MTCC 442 and *V. cholera* MTCC 3904 as compared to standard drugs Gentamycin, Amikacine.

Compound FC3, exhibited excellent efficacy (zone of growth inhibition 63.98, 63.93 mm) against tested *S.aureus* MTCC 3160, *B.cereus* MTCC 430. Whereas in case of *B. cereus* MTCC 430, FC7 (32.22 mm) nearly same to Gentamycin (32.18 mm). In case of *S. aureus* MTCC 3160 and *B. cereus* MTCC 430, a number of compounds

(FC2, FC3, FC6) displayed inhibitory activity (40.93, 63.98, 40.15 mm) better than that of Gentamycin (32.0-34.0mm), while compounds (FC2, FC3, FC6, FC7) have shown better activity (40.93, 63.98, 40.15, 32.22 mm) than satandard drug Amikacin (27.0-29.0mm). It is evident from table that compound FC3 displayed effective activity profile (zone of growth inhibition 63.98 mm) against all the two Gram-positive bacteria.

The zone of growth inhibition of compounds FC7 (29.0mm, 31.0mm) for *E. coli* MTCC 442 nearly equal to control drug Gentamycin whereas in case of *V. cholera* MTCC 3904. In case of *E. coli* MTCC 442 and *V. cholera* MTCC 3904, a number of compounds (FC2, FC3, FC6, FC7) displayed inhibitory activity (39.13, 63.89, 39.13, 32.3 mm) better than that of Gentamycin (29.01-30.11 mm). While compound (FC2, FC3, FC6, FC7) have shown better activity (39.13, 63.89, 39.13, 32.3 mm) than standard drug Amikacin (25.12-26.23 mm).

Compounds FC8 (14.13, 14.56, 13.39, 9.17 mm) showed minimum zone of growth inhibition compared to standard drug Gentamycin (34.17, 32.18, 30.11, 29.01 mm) and Amikacin (29.11, 27.98, 25.12, 26.23 mm) against all Gram-positive bacteria *S. aureus* MTCC 3160 and *B. cereus* MTCC 430 and Gram-negative bacteria *E. coli* MTCC442 and *V.cholerae* MTCC 3904

From the table 2 it is clear that the tested flavonoids derivatives many compounds exhibited varying levels of antibacterial activity against all the Gram-positive as well as Gram-negative bacteria. It has also been observed, from the table that Gram-positive bacteria were more susceptible towards the newly synthesized series of compounds (FC1-FC8 series) as compared to Gram-negative bacteria.

Table 2: Antibacterial screening data for Flavonoid derivatives.

Compound	Zone of Inhibition (mm)			
	Gram-Positive		Gram -Negative	
	SA[b]	BC[c]	EC[d]	VC[e]
FC1	22.83±0.12	-	22.39±0.07	19.91±0.16
FC2	40.93±0.20	40.91±0.18	39.13±0.13	39.09±0.02
FC3	63.98±0.11	63.93±0.31	63.12±0.18	63.89±0.09
FC4	15.93±0.35	14.92±0.39	-	14.13±0.15
FC5	22.44±0.16	23.97±0.26	22.14±0.19	21.49±0.47
FC6	40.09±0.9	40.15±0.16	39.13±0.27	38.94±0.03
FC7	32.12±0.31	32.22±0.07	31.13±0.09	32.03±0.22
FC8	14.13±0.90	14.56±0.42	13.39±0.28	9.17±0.03
Gentamycin[a]	34.17±0.28	32.18±0.98	30.11±0.12	29.01±0.04
Amikacin[a]	29.11±0.18	27.98±0.91	25.12±0.26	26.23±0.19

[a] Antibacterial activity of the synthesized compounds was compared with the standard antibacterial drugs Gentamycin and Amikacine.; [b]SA: Staphylococcus aureus MTCC 3160; [C] BA: Bacillus cereus MTCC 430; [d]EC: Escherichia coli MTCC 442; [e]VC: Vibrio cholerae MTCC 3904.

The result for the antibacterial activity of the synthesized compounds against *S. aureus* MTCC 3160, *B. cereus* MTCC 430, *E. coli* MTCC 442, *V. cholerae* MTCC 3904 are graphically represented in figures 2 and 3.

From the figures 2 and 3, it is clear that most out of the synthesized compounds gave promising results compared to the reference drugs Gentamycin and Amikacin against the tested Gram-positive and Gram-negative bacteria.

The negative % inhibition values of FC2, FC3, FC6 and FC7 for various bacterial strains have better antibacterial activity profile of these derivatives as compared to the standard drugs Gentamycin and Amikacin. The % inhibition +100 indicates that there was no antibacterial activity. The decreasing order of antibacterial activity is –

FC3> FC2> FC6> FC7> FC5> FC1> FC8> FC4> FA6.

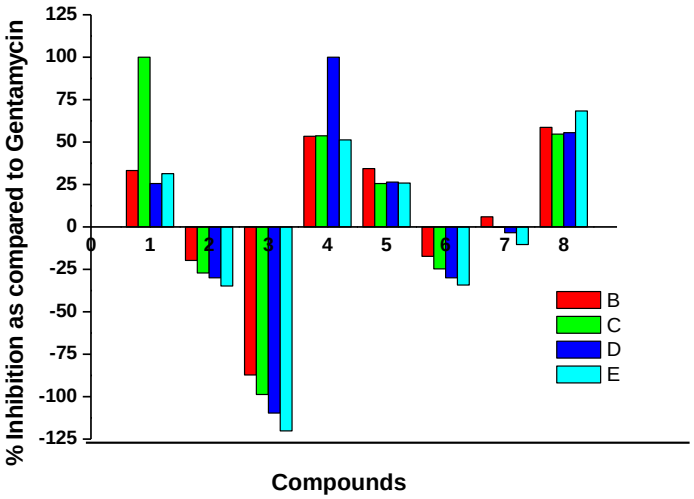


Fig. 2: Percent (%) inhibition values.

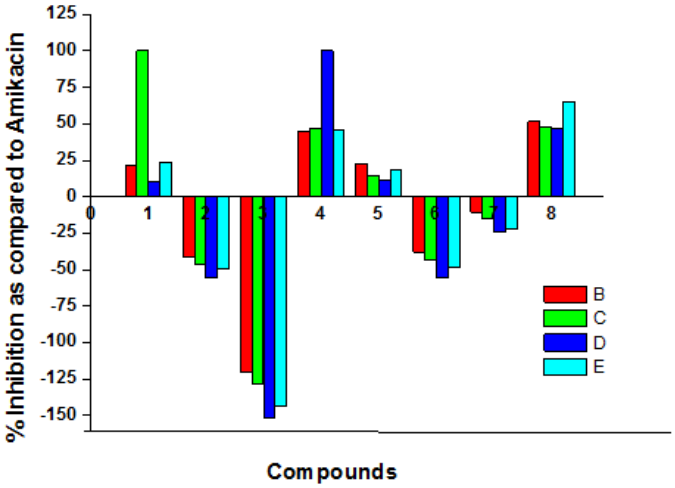


Fig. 3: Percent (%) inhibition values.

CONCLUSION

A number of flavones derivatives (FC1-FC8) were synthesized as potential antibacterial agents from the method were used in this study result concluded that compounds FC3, FC2, FC6 and FC7 shown good antibacterial activity against all the tested bacterial strains. The compound FC3 has shown maximum inhibitory activity among the all synthesized derivatives compared to standard antibacterial drugs Gentamycin and Amikacin against all of the Gram-positive and Gram-negative bacteria. Here it was also clear that substituents on the flavones skeleton are responsible for enhance their antibacterial activity.

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