CHAPTER IV

ANTIMICROBIAL ACTIVITY OF FLAVONOIDS

IV.1. Introduction

Many plants and their isolates have been screened for their possible antimicrobial activity[177-178]. Anthocyanians[179], dihydrochalcone[180], flavanone[181], isoflavones[182] and flavonoids[183-185] have been reported to possess antimicrobial activity.

Many factors are involved in testing the antimicrobial activity of a compound, viz. the size of the inoculum, the nature of the culture medium, the concentration of the test compound, the pH of the medium, the temperature and time of incubation. The three important methods employed for antimicrobial sensitivity of a drug / compound are (i) Disc Diffusion Technique[186] (ii) Serial Dilution Technique[187] and (iii) Ditch plate Technique[188].

The principle involved in the disc diffusion technique is to prepare a concentration gradient of the drug in a nutrient medium and observe the growth of the bacteria that is seeded in the medium after an incubation period[189]. The clear zone of growth inhibition around the disc is the result of two processes, diffusion of the drug and growth of the bacteria. As the antimicrobe diffuses

through the agar medium from the edge of the disc, its concentration progressively diminishes to a point where it is no longer inhibitory for the organism. The size of this area of suppressed growth or the zone of inhibition is determined by the concentration of antimicrobe present in the area and the susceptibility of the test isolate. Therefore the diameter of the inhibition zone denotes the relative susceptibility of the test microorganism to a particular antimicrobe.

Depending on the measured value of complete inhibition diameter of the circle measured in mm., the antimicrobe is classified into the following types.

Inhibition zone diameter	Type of antimicrobe
> 13 mm	Highly sensitive or susceptible
8.13 mm	Moderately sensitive or intermediate.
< 8 mm	Resistance

The term 'susceptible' implies that an infection caused by the strain tested may be expected to respond favourably to the indicated antimicrobial agent for that type of infection and pathogen. 'Resistance' strains are not inhibited completely by therapeutic concentrations. 'Intermediate' implies that strains may respond to unusually high concentrations of the agent, resulting from high dosage.

During the study, six different monohydroxyflavones, their acetyl and glucosyl derivatives were tested for their antimicrobial activity with drug Norfloxacin(NF) as reference drug. The test organisms chosen were Staphylococcus aureus and Streptococcus pneumoniae of the Gram - positive bacteria group and Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa of the Gram-negative group.

IV.2. Experimental

Circular discs (6.25 mm dia) were prepared from Whatman No.1 filter paper and sterilised by dry heat at 140^{o}C for one hour in batches of 100 in screw-capped bottles. Individual flavonoids (1000 µg in 1ml of DMF) were added to each bottle of 100 discs. Each disc contain 10 µg of the test compound when the complete volume was absorbed.

Agar (pH 7.2 - 7.4 at room temperature) was prepared[190] and immediately cooled to 50°C in a water bath after removal from the autoclave. The cooled medium was then poured into sterile petriplates to an uniform depth of 4 mm After being allowed to solidify at room temperature, the plates were used the same day or refrigerated at 2 to 8°C for no longer than seven days. Just prior to the use of the medium, the plates were placed in a 35°C incubator for about twenty minutes, until the excess surface moisture has evaporated.

Using sterile fine pointed forceps the selected discs were placed on the inoculated plate and pressed firmly into the agar. The discs were distributed evenly in such a manner as to be not closer than 15 mm from the edge of the petridish and in such a way that no two discs were closer than 24 mm from centre to centre. Once a disc had been placed, it should not be moved, since some diffusion of the drug occurs almost instantaneously. The plates were inverted and placed in the 35°C incubator within 15 minutes after application of the discs.

After incubation, the relative susceptibility of the organism to the antimicrobe is demonstrated by a clear zone of growth inhibition around the disc containing the flavonoid.

IV.3. Results and Discussion

The average results of the three tests subjected to Q test are presented in Table IV 1.

The values in bracket indicate the inhibition zone diameter in mm.

Results show that there exists a pattern of selective toxicity in the flavonoids towards Gram-positive bacteria. Of the 18 flavonoids tested for antimicrobial activity, seven were active against organism 1, six against organism 2, five against organism 3, three against organism 4 and three against organism 5.

The selective bacterial activity of flavonoids may be attributed among other things to the wall thickness of bacterial cells. Walls of Gram-positive organisms in general are thin whereas those of Gram-negative groups are much thicker. These results are in agreement with the conclusion of the earlier researchers about the Gram-positive bacteria as being selectively inhibited[191].

TABLE IV 1

Antimicrobial Activity of Monohydroxyflavones their Acetyl and Glucosyl Derivatives.

No.	Test Organism	Antimicrobe Susceptible	Intermediate
1.	Staphylococcus aureus	3(16) NF(26)	2G(11) 3A(8) 3G(8) 5A(12) 5(8) 7A(8)
2.	Streptococcus pneumoniae	3(14) 3A(14) NF(30)	3G(9) 5A(11) 6(8) 7(8)
3.	Escherichia coli	3(22) 4A(22) 6A(17) NF(24)	6G(13)
4.	Klebsiella pneumoniae	NF(25)	2(8) 3G(8) 6A(9)
5.	Pseudomonas aeruginosa	2A(15) 7(16) NF(26)	6G(11)