IJPSR (2012), Vol. 3, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 August, 2012; received in revised form 15 October, 2012; accepted 30 November, 2012

SYNTHESIS AND *IN VITRO* ANTIPLAQUE ACTIVITY OF CHALCONE, FLAVONOL AND FLAVANOL DERIVATIVES

Vijayalaxmi Chavan Patil

Government College of Pharmacy, Aurangabad-431005, Maharashtra, India

Keywords:
Chalcone, Hydroxyflavones, Flavonols, Antiplaque, *Streptococcus mutans*

Correspondence to Author:

Vijayalaxmi Chavan Patil

Government College of Pharmacy, Aurangabad-431005, Maharashtra, India

E-mail: vijayalaxmi.pharma@gmail.com

ABSTRACT

Synthesis of chalcone derivatives was carried out by aldol condensation, these 2'-hydroxychalcones were cyclized to 3-hydroxyflavone and 2, 3-dihydroflavan-3-ol derivatives and we also report here there *in vitro* antiplaque activity. Most of the synthesized compounds were found to be active against *Streptococcus mutans.* Activity of two 3-hydroxyflavones was found to be higher than that of their corresponding 2, 3- dihydroflavan-3-ol derivatives and chalcone analogues. Investigated compounds having hydroxy group substituents in ring-B exhibited enhanced activity and the presence of electronegative groups in the studied compounds showed a direct relationship to the antiplaque activity.

INTRODUCTION: Flavonoids are yellow color pigments and being named so as in Latin Flavus means yellow colour. Structurally, flavonoids are derivatives of benzopyrone, a group of aromatic oxygen-bearing heterocyclic compounds. Flavonoids are chromene which has basic ring system of benzo-4-pyranone [1].

All these Flavonoid derivatives are associated with free radical scavenging activity through various mechanisms like Direct radical scavenging, Inhibition of nitric oxide synthesis, Xanthine oxidase inhibition, Leukocyte immobilization inhibition, Direct inhibition of lipid peroxidation etc [2].

Flavonoid derivatives shows cardioprotective, gastroprotective, anti-inflammatory activity, Anti-tumor, Antithrombogenic, Antiviral, Anti-osteoporotic, Antimicrobial, Antiprotozoal, Antiplaque activities & also prevents dementia [3-11].

Chalcones are yellow pigments known as precursors of many naturally occurring pigments such as flavones. Chalcones are substituted benzalacetophenone derivatives, due to the presence of the reactive keto vinylenic group, chalcones and their analogues have been reported to be antiulcerative, anti-inflammatory, anti-angiogenic, analgesic, anticancer and antioxidant [12, 13].

The micro-organism responsible for tooth decay is *Streptococcus mutans*, it is gram positive anaerobic bacteria. It causes yellow plaque deposition on teeth, and further deteriorate the condition by causing gingivial inflammation i.e. Gingivitis, it is an inflammation of gums i.e. Gingiva around the teeth. In general terms the gingivitis is refered to the inflammation induced by bacterial biofilms adhered to tooth surface known as Plaques.

Green tea has been extensively examined, as a useful plant that contains flavonoids, in the control of plaque-related diseases. With respect to dental caries, extracts have been shown to kill *Streptococcus mutans* and *Streptococcus sobrinus*.

Inhibition of bacterial adherence, acid production, and glucosyl transferase activity (which is involved in extracellular polysaccharide formation), have all been demonstrated. Animal and human trials also support the use of green tea in the control of dental caries [14].

Literature states that plant flavonoids in toothpastes are responsible for antiplaque action and works against tooth decay. Plaque-related diseases are probably the most common bacterial diseases occurring in man. Flavonoid derivatives which posses hydroxylated phenolic nucleus where the C6-C3 unit is linked to an aromatic ring, demonstrates a broad range of antimicrobial activity. For example, catechins as found in green tea [15]. Guaijaverin isolated from *Psidium guajava* Linn. Shows anti- *Streptococcus mutans* activity [16].

In nature, flavonoids are available but their use as drug is limited by high cost of processing. There are some marketed products of natural flavonoids from plant sources except a few for various activities like antiinflammatory, anticancer, antiviral, antimicrobial, muscle relxant, antiageing, antialzimer's and other neurodegenerative diseases. Widespread use of flavonoids is hindered because of its limited availability, lengthy, tedious, time consuming and uneconomic isolation, separation, purification, and structural modification techniques.

Thus, goal of this work is to synthesize flavonoid derivatives starting from commonly available raw materials and its evaluation for Antiplaque activity.

In present investigation, 2'-hydroxychalcone derivatives were synthesized by Aldol condensation, in this reaction hydroxy-acetophenone derivatives were treated with aromatic aldehyde in presence of strong base like NaOH.

Further, flavonol derivatives were synthesized by Algar Flynn Oyamada reaction, the 2'-hydroxy chalcones were converted to 3-hydroxy flavones (flavonol) and 2,3-dihydroflavan-3-ol (flavanol) by cyclization. All the reactions are shown in **Figure 1-3**.

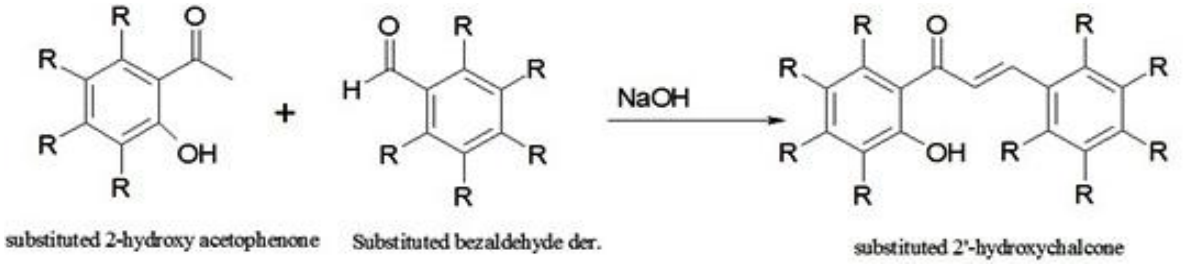


FIG. 1: SYNTHESIS OF 2'-HYDROXYCHALCONE DERIVATIVES

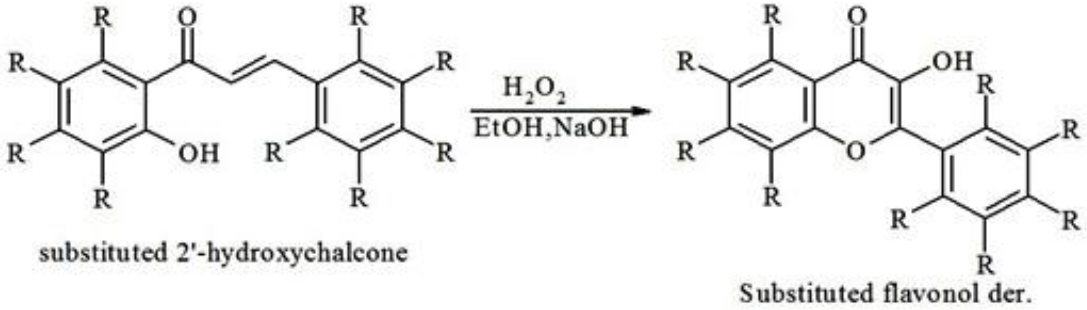


FIG. 2: SYNTHESIS OF 3-HYDROXY FLAVONES (FLAVONOL) DERIVATIVES

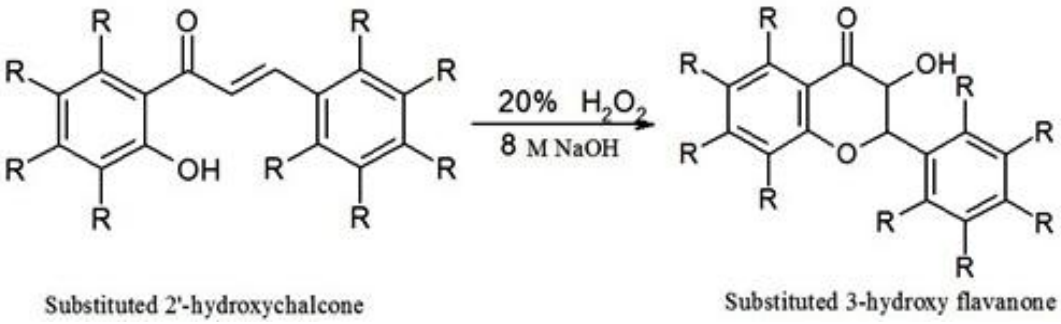


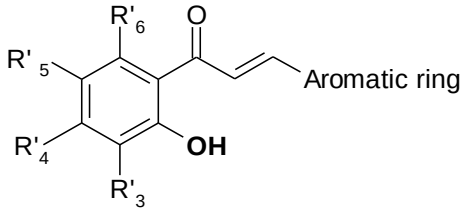
FIG. 3: SYNTHESIS OF 2,3-DIHYDROFLAVAN-3-OL (FLAVANOL) DERIVATIVES

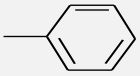
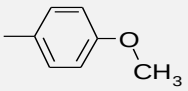
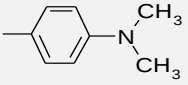
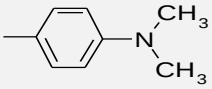
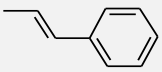
2'-hydroxychalcone derivatives (C1-C5) were synthesized by following procedure [17]. Equimolar quantities of aromatic aldehyde derivatives (0.01 mol) and acetophenone derivatives (0.01 mol) as shown in **Table 1** were dissolved in minimum amount of alcohol. Sodium hydroxide solution (0.02 mol) was added slowly and the mixture stirred for 2hr until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol. The completion of the reaction was monitored by TLC. Yields, melting points & Rf values of C1-C5 are reported in **Table 2** .

TABLE 1: RAW MATERIAL USED FOR SYNTHESIS OF 2'-HYDROXYCHALCONE DERIVATIVES

Hydroxy acetophenone	Aromatic aldehyde	Chalcone
2-hydroxyacetophenone	Benzaldehyde	C1
2-hydroxyacetophenone	Anisaldehyde	C2
2-hydroxyacetophenone	p-N,N-dimethylamino benzaldehyde	C3
2,4-dihydroxyacetophenone	p-N,N-dimethylamino benzaldehyde	C4
2-hydroxyacetophenone	Cinnamaldehyde	C5

TABLE 2: PHYSICAL CHARACTERISATION DATA OF SYNTHESIZED 2'-HYDROXYCHALCONE DERIVATIVES



Compound code	Compound Name	Substituents			Melting point	% Yield	Rf
		X	R'3, R'5, R'6	R'4			
C1	1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one		H	H	89^{0}C	80.4 %	0.69
C2	1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one		H	H	92^{0}C	91%	0.8
C3	3-[4-(dimethylamino)phenyl]-1-(2-hydroxyphenyl)prop-2-en-1-one		H	H	160^{0}C	94.20%	0.73
C4	1-(2,4-dihydroxyphenyl)-3-[4-(dimethylamino)phenyl]prop-2-en-1-one		H	OH	190^{0}C	90.03%	0.59
C5	1-(2-Hydroxy-phenyl)-5-phenyl-penta-2,4-dien-1-one		H	H	150^{0}C	88.8%	0.69

C1= $C_{15}O_2H_{12}$, IR (KBr cm^{-1}) 1644(C=O), 3028(O-H), 1300-1400(C=C), 600-900(C-H aromatic), UV λmax (nm) 373(1.284), 249(0.612), MS (GC-MS) m/z 223(M+), 147($C_9O_2H_6^+$), 207 ($C_{15}H_{12}O$), 120 ($C_6H_5O_2$), 103 ($C_8H_7^+$)

C2 = $C_{16}H_{14}O_3$, IR (KBr cm^{-1}) 1774 (C=O), 3069 (O-H), 1300-1400(C=C), 600-900(C-H aromatic), UV λmax (nm) 317 (0.644), 251(0.559), MS (GC-MS) M/Z 268(M), 253 ($C_{15}H_9O_4$), 85($C_3H_2O_3^+$)

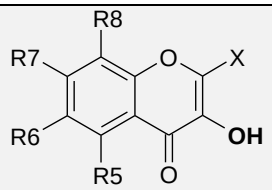
C3 = $C_{17}H_{17}O_2N$, IR (KBr cm^{-1}) 1604 (C=O), 3074 (O-H), 3301(C-N), 1300-1400(C=C), 600-900(C-H aromatic), UV λmax (nm) 346 (1.276), 247(0.479)

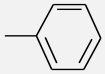
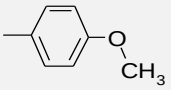
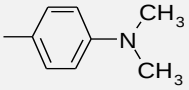
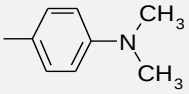
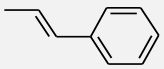
C4 = $C_{17}H_{17}O_3N$, , IR (KBr cm^{-1}) 1662 (C=O), 3092 (O-H), 1300-1400(C=C), 600-900(C-H aromatic), UV λmax (nm) 360 (2.004), 232(0.779)

C5 = $C_{17}H_{14}O_2$, IR (KBr cm^{-1}) 1634 (C=O), 3065 (O-H), 1300-1400(C=C), 600-900(C-H aromatic), UV λmax (nm) 346 (1.276), 247(0.479)

Flavonol derivatives (F1-F5) were synthesized by following procedure [18]. To a suspension of chalcone (0.01mole) in 85ml ethanol was added to 10 ml 20% aqueous sodium hydroxide with stirring, followed by careful addition of 18ml 20% hydrogen peroxide over a period of 0.5hr. The reaction mixture was stirred for 3 hrs. at 30°C and poured onto crushed ice containing 5N HCl. The precipitate was filtered, washed, dried and crystallized from chloroform: methanol [9:1] . Yields, melting points & Rf values of F1-F5 are reported in **Table 3**.

TABLE 3: PHYSICAL CHARACTERIZATION DATA OF SYNTHESIZED 3-HYDROXY FLAVONES DERIVATIVES



Compound code	Compound Name	Substituents			Melting point	% Yield	Rf
		X	R5, R6, R8	R7			
F1	3-hydroxy-2-phenyl-4H-chromen-4-one		H	H	169°C	64%	0.57
F2	3-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one		H	H	229°C	76 %	0.65
F3	2-[4-(dimethylamino)phenyl]-3-hydroxy-4H-chromen-4-one		H	H	196°C	82%	0.69
F4	2-[4-(dimethylamino)phenyl]-3,7-dihydroxy-4H-chromen-4-one		H	OH	297°C	74.8%	0.50
F5	3-hydroxy-2-[2-phenylethenyl]-4H-chromen-4-one		H	H	265°C	72%	0.56

F1= $C_{15}H_{10}O_3$, IR (KBr cm^{-1}) 1605 (C=O), 3193 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 351 (2.146), 249(1.955), MS (GC-MS) M/Z 237(M+), 77(C_6H_6)

F2= $C_{16}H_{12}O_4$, IR (KBr cm^{-1}) 1606 (C=O), 3225 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 360 (1.902), 259(1.234)

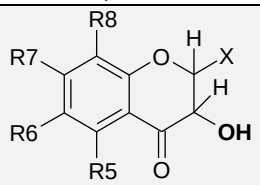
F3 = $C_{17}H_{15}O_3$ N, IR (KBr cm^{-1}) 1603 (C=O), 3305 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), MS (GC-MS) M/Z 281(M), 105($C_7H_5O^+$)

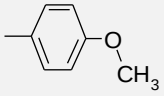
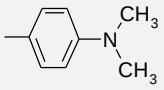
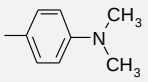
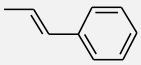
F4 = $C_{17}H_{15}O_4N$, IR (KBr cm^{-1}) 1678 (C=O), 3049 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 338 (1.796), 240(0.427)

F5 = $C_{17}H_{12}O_3$, IR (KBr cm^{-1}) 1590 (C=O), 3060 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 437(0.962), 256(1.582)

2'-hydroxy chalcone derivatives (C1-C5) were cyclised to 2,3-dihydroflavan-3-ol derivatives (S1-S5) by following procedure. A suspension of a mixture of powdered 2'-hydroxychalcone (0.45 mmol), an aq. NaOH solution (8 M, 1.0 ml) and a 30% hydrogen peroxide solution (0.25 ml) was stirred at room temperature for 2 h. The crude product was filtered off, washed with water and dried in a desiccator to give flavanol. Recrystallization of the crude product from Methanol. Yields, melting points & Rf values of S1-S5 are reported in **Table 4**.

TABLE 4: PHYSICAL CHARACTERISATION DATA OF SYNTHESIZED 2, 3-DIHYDROFLAVAN-3-OL DERIVATIVES



Compound code	Compound Name	Substituents			Melting point	% Yield	Rf
		X	R5, R6, R8	R7			
S1	3-hydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one		H	H	178^{0}C	69%	0.73
S2	3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one		H	H	206^{0}C	72.9%	0.87
S3	2-[4-(dimethylamino)phenyl]-3-hydroxy-2,3-dihydro-4H-chromen-4-one		H	H	210^{0}C	71.7%	0.76
S4	2-[4-(dimethylamino)phenyl]-3,7-dihydroxy-2,3-dihydro-4H-chromen-4-one		H	OH	248^{0}C	74.1%	0.64
S5	3-hydroxy-2-[-2-phenylethenyl]-2,3-dihydro-4*H*-chromen-4-one		H	H	285^{0}C	74.1%	0.69

S1= $C_{15}H_{12}O_3$, IR (KBr cm^{-1}) 1583 (C=O), 3070 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 322(1.469), 227(0.923),

S2= $C_{16}H_{14}O_4$, IR (KBr cm^{-1}) 1572 (C=O), 3051 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 339(1.313), 242(0.391)

S3= $C_{17}H_{17}O_3$ N , IR (KBr cm^{-1}) 1601 (C=O), 3051 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 345(0.743), 250(0.177)

S4= $C_{17}H_{17}O_4N$, IR (KBr cm^{-1}) 1664 (C=O), 3183 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 341(2.005), 241(0.419), MS (GC-MS)

S5= $C_{17}H_{14}O_3$, IR (KBr cm^{-1}) 1678 (C=O), 3049 (OH), 1300-1400 (C=C), 600-900 (C-H aromatic), UV λmax (nm) 353(1.70), 250(0.98)

Reactions and purity of compounds were monitored by thin layer chromatography (silica gel G60) using chloroform: methanol [9:1] solvent system and the spots were identified by iodine vapor chamber. Melting points were determined in open capillary using paraffin bath and are uncorrected. Infra red spectra was recorded using FT-IR instrument in range 400-4000 cm^{-1}, Diffraction reflectance scanning (DRS) technique was used to record the spectra. Mass spectral analysis was done in GC/MS Q2010 instrument by mass spectrometry technique using DI probe.[19]

Synthesized compounds were tested for their antibacterial activity by adopting agar well diffusion method. Using Streptococcus mutans Muller Hinton Agar (MHA) plates were used. Wells were created in the medium with the help of a sterile metallic borer at appropriate distances.

Test samples of 1000 µg/ml were poured into each well and the plates were incubated at 37 ^{0}C for 24 h. The results, in terms of inhibition zones, were noted and reported in Table 5 and shown in figure 4.

TABLE 5: ZONE OF INHIBITION

Compound code	Zone of inhibition
C1	12mm
C2	10 mm
C3	13mm
C4	8mm
C5	NA
F1	15mm
F2	6mm
F3	12mm
F4	6mm
F5	6mm
S1	10mm
S2	6mm
S3	10mm
S4	12mm
S5	5mm

FIG. 4: PHOTOGRAPHS OF ZONE OF INHIBITION STUDY

On observation of positive results of antimicrobial activity, the Minimum Inhibitory Concentration of synthesized compounds were determined by tube dilution method and reported in **Table 6.**

Study indicated the requirement of highest concentration of 2'-hydroxy chalcone derivatives than 2'-hydroxy chalcone derivatives than 3-hydroxyflavone derivatives to inhibit the *Streptococcus mutans.* In other words MIC of 3-hydroxyflavone derivatives is lower than that of 2'-hydroxy chalcone derivatives. At the end of study it was concluded that C5 [1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is inactive against *Streptococcus mutans*.

All 3-hydroxyflavone derivatives (F1-F5) showed their MIC in range of 250-125 µg/ml, 2'-hydroxy chalcone derivatives (C1-C5) showed inhibition at 250 µg/ml and 2,3-dihydro-flav-3-ol derivatives (S1-S5) showed inhibition at higher concentration of 500-1000 µg/ml. These results are compared in **Figure 5-11**.

TABLE 6: MIC OF CHALCONE AND FLAVONOID DERIVATIVES WITH *STREPTOCOCCUS MUTANS*

Compound code	% Transmittance				
	1000	500	250	125	62.5
C1	38	36	**33**	15	13
C2	26	27	**28**	18	20
C3	20	**28**	19	18	9
C4	34	37	**40**	25	22
C5	5	10	8	9	43
F1	32	34	**36**	12	10
F2	33	36	**25**	15	14
F3	33	35	**28**	19	20
F4	32	33	30	**27**	12
F5	34	39	**35**	27	23
S1	**46**	24	21	25	17
S2	**34**	15	18	15	14
S3	59	**45**	23	27	21
S4	43	**46**	32	30	26
S5	**54**	16	13	18	15

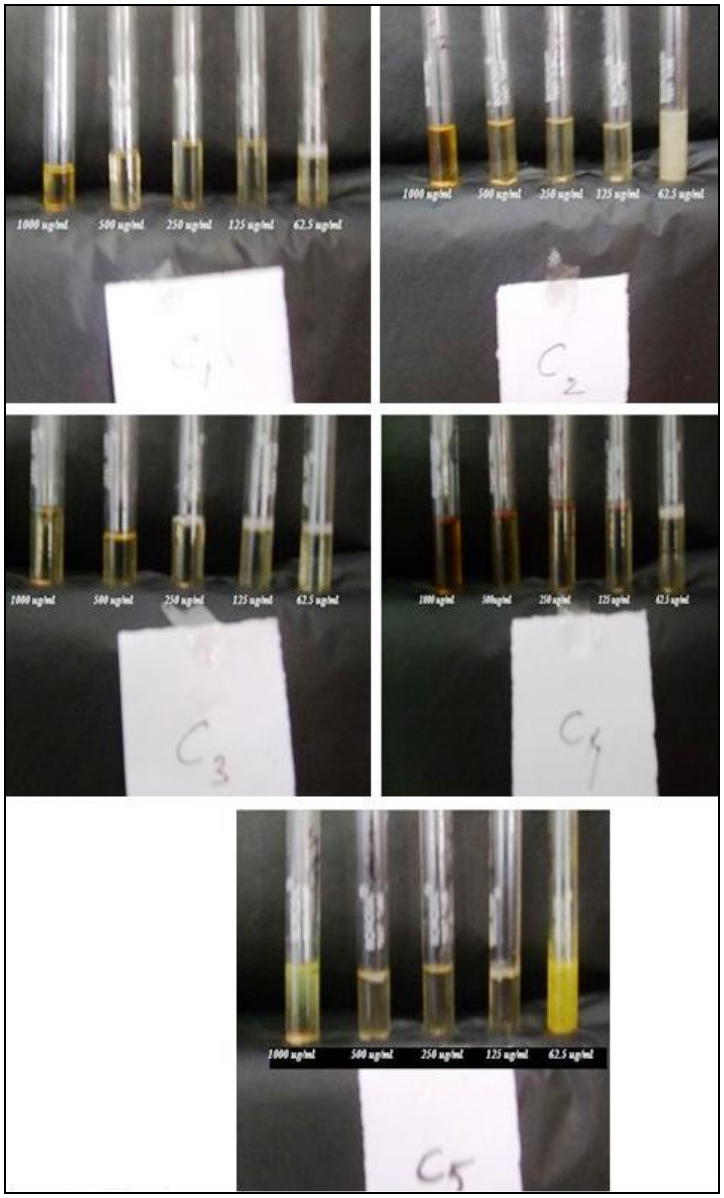


FIG. 5: PHOTOGRAPHS OF MIC OF 2'-HYDROXY CHALCONE DERIVATIVES

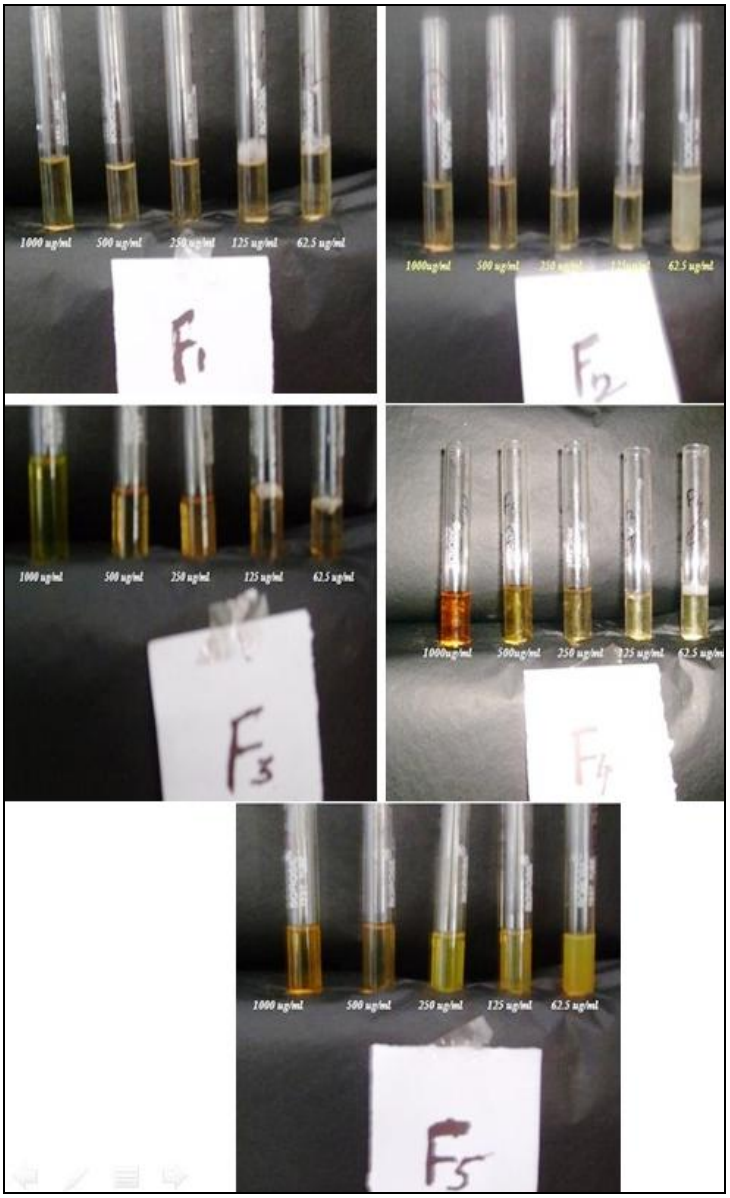


FIG. 7: PHOTOGRAPHS OF MIC OF 3-HYDROXYFLAVONE DERIVATIVES

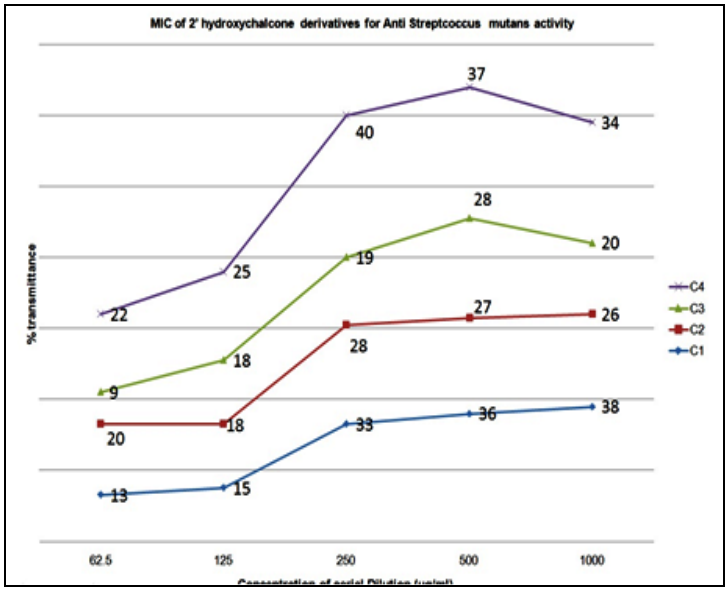


FIG. 6: MIC OF 2'-HYDROXY CHALCONE DERIVATIVES

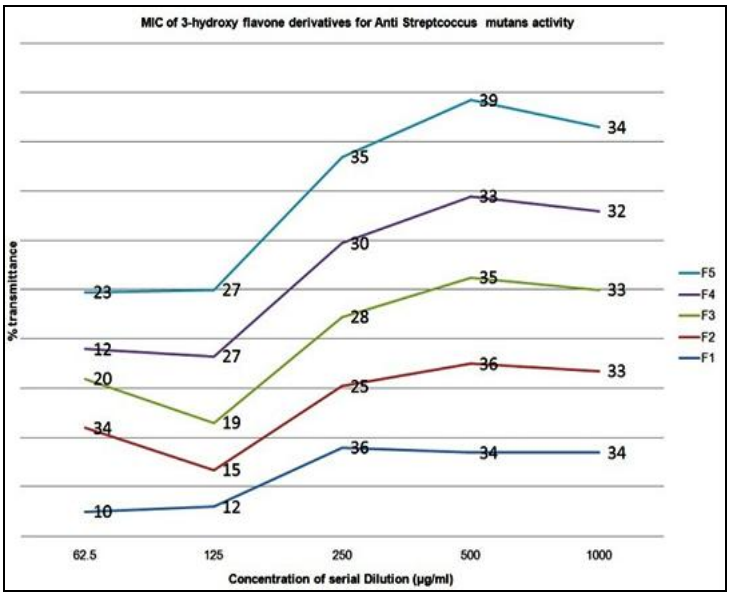


FIG. 8: MIC OF 3-HYDROXYFLAVONE DERIVATIVES

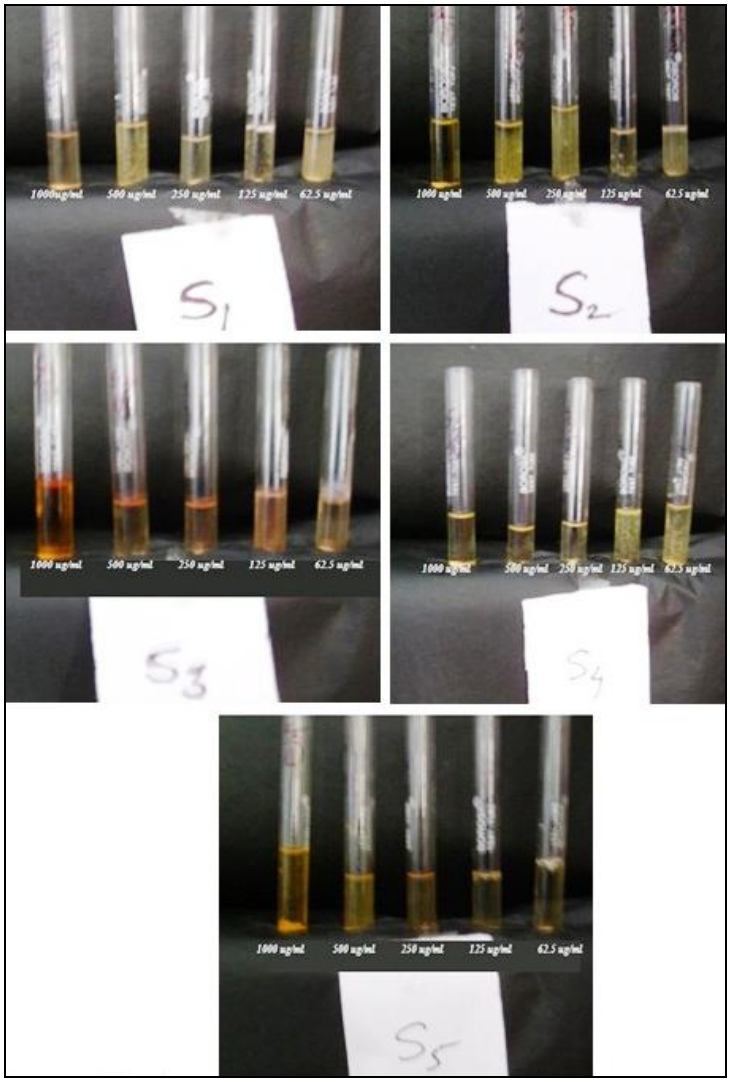


FIG. 9: PHOTOGRAPHS OF MIC OF 2,3-DIHYDRO-FLAV-3-OL DERIVATIVES

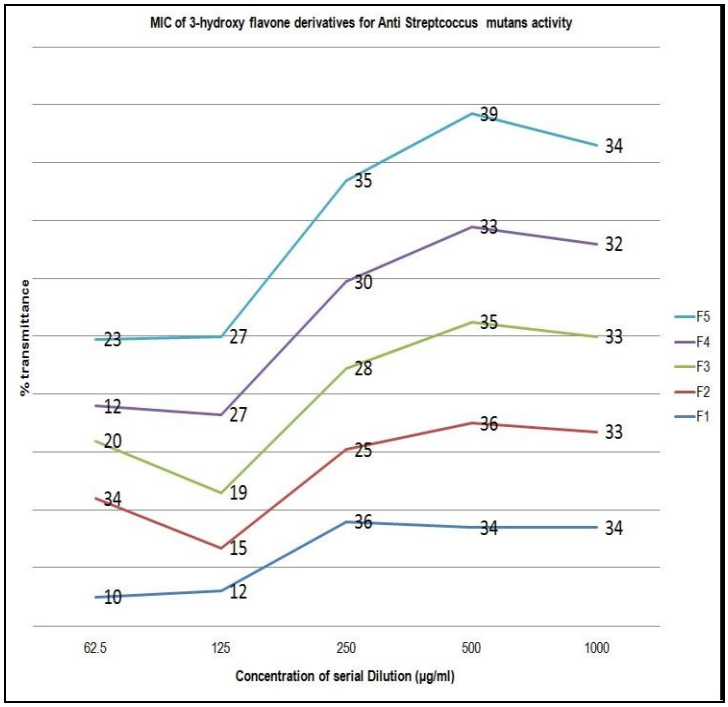


FIG. 10: MIC OF 2,3-DIHYDRO-FLAV-3-OL DERIVATIVES

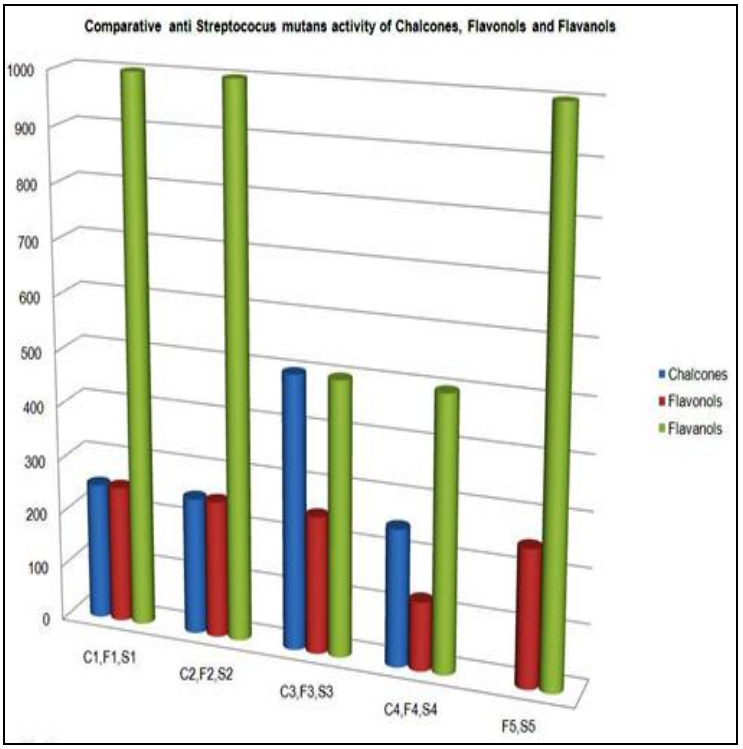


FIG. 11: COMPARATIVE ANTI STREPTOCOCCUS MUTANS ACTIVITY OF CHALCONES, FLAVONOLS AND FLAVANOLS.

2'-hydroxychalcone, 3-hydroxyflavone & 2,3-dihydro-flav-3-ol derivatives can be synthesized under available laboratory conditions and were confirmed by physicochemical and spectral analysis.

The result of antimicrobial study against plaque causing microorganism Streptococcus mutans shows that 3-hydroxy flavone derivatives are more active than corresponding 2-hydroxy chalcone & 2,3-dihydro-flav-3-ol derivatives.

Simple and feasible method of synthesis of flavonoid derivatives and evalution of antiplaque activity provided a path to explore the fascinating class of active compound.

These findings about the synthetic methods of flavonoids and their antiplaque activity is highly encouraging and many more flavonoids should be synthesized to obtain most useful once, and the work in this direction will be continued in future.

ACKNOWLEDGEMENT: The author wish to acknowledge Dr. V.K. Mourya, Principal, Government College of Pharmacy, Aurangabad for his idea of present research work, facilities provided for synthesis, analysis & activity evaluation.

REFERENCES:

1. Beecher GR: Overview of Dietary Flavonoids: Nomenclature, Occurrence and Intake. The American Society for Nutritional Sciences 2003; 133:3248S-3254S.
2. Gustavo LL, Moreno L, Cogolludo A, Galisteo M, Ibarra M, Duarte J: Nitric Oxide (NO) Scavenging and NO Protecting Effects of Quercetin and Their Biological Significance in Vascular Smooth Muscle. Molecular Pharmacology 2004; 65(4):851-859.
3. Ares: Use of flavone derivative for gastroprotection. 1995; US patent: US 5399584.
4. Zayachkivska OS , Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR: Gastroprotective effects of flavonoids in plant extracts. Journal of Physiology and Pharmacology 2005; 56 (Suppl 1):219-231.
5. Kanadaswami C, Lee LT, Lee PP, Hwang JJ, Chun K, Huang YT: The Antitumor Activities of Flavonoids. in vivo 2005; 19: 895-910.
6. Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. Bioscience Biotechnololgy and Biochemistry 1999; 63(10):1787-90.
7. Serafini M, Peluso I, Raguzzini A: Flavonoids as anti-inflammatory agents. The Proceedings Nutrition Society 2010; 69(3):273-8.
8. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F: Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine 1995; 155(4)381–386.
9. Wang HK, Xia Y, Yang ZY, Natschke SL, Lee KH: Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. Advances in Experimental Medicine and Biolology 1998; 439:191-225.
10. Creuzet MH, Feniou C, Guichard F, Prat G, Mosser J, Pontagnier H: 3-hydroxyflavones: their preparation and therapeutic application 1986; US patent: US 4591600.
11. Lawlor TM: Oral care compositions. 2004; US patent: US 6706256
12. Komazawa Y, Takeda S, Hosaka K, Mitsuhashi H, Watanabe T: Anti-ulcer agent comprising chalcone derivative. 1993; US patent: US 5234951
13. Bombardelli E, Valenti P: Chalcones having antiproliferative activity. 2002; US patent: US 6423740
14. Allaker RP, Douglas CW: Novel anti-microbial therapies for dental plaque-related diseases. International Journal of Antimicrobial Agents 2009; 33(1): 8–13.
15. Anderson OM, Markham KR: Flavonoids:Chemistry. Biochemistry and Applications, CRS Press, Taylor and Fransis group: 2006.
16. Prabu GR, Gnanamani A, Sadulla S: Guaijaverin- a plant flavonoid as potential antiplaque agent against Streptococcus mutans. J Appl Microbiol 2006;101(2):487-95.
17. Kalirajan R, Sivakumar SU, Jubie S, Gowramma B and Suresh B: Synthesis and Biological evaluation of some heterocyclic derivatives of Chalcones. Int.J. Chem Tech Res 2009; 1(1):27-34.
18. Kurzwernhart A, Kandioller W, Bartel C, Bächler S, Trondl R, Muhlgassner G : Targeting the DNA-topoisomerase complex in a double-strike approach with a topoisomerase inhibiting moiety and covalent DNA binder, article downloaded from http://www.rsc.org/suppdata/cc/c2/c2cc31040f/c2cc31040f.pdf on 11 Aug 2012.
19. Markham KR: Techniques of flavonoid identification. Academic press, London, 1982; 36-49.

How to cite this article:
Patil VC: Synthesis and *In-vitro* Antiplaque Activity of Chalcone, Flavonol and Flavanol Derivatives. *Int J Pharm Sci Res*. 3(12); 5006-5014.