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Designing and Synthesis of Flavonoids Derivatives and Screening of their Antimicrobial Activity

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ABSTRACT

Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). The *streptococcus mutans* is a bacteria that found in the human mouth cavity. This bacterial strain produces plaque and acids that break down tooth enamel and cause dental caries. Gram positive cocci, facultatively anaerobic bacteria that forms rod-like chains. the chemical reaction of 2- hydroxyacetophenones with aromatic acylchloride occurs to form 1,3-diketones. This rearrangement reaction proceeds via enolate formation followed by acyl transfer. Then it cyclises into flavone.[13] As the same of above scheme can be worked out as 2- Methoxybenzoyl Chloride is prepared by reaction of 2-methoxybenzoic acid with Thionyl chloride and DMF. 2-Methoxybenzoyl Chloride then added to mixture of 2- hydroxyacetophenone and pyridine, 2-[(2-Methoxybenzoyl)oxy]acetophenone thus obtained is treated with pyridine and KOH which gives1-(2-Hydroxyphenyl)-3-(2-methoxyphenyl)-propan1,3-dione. The result of study indicated that C5 [1-(2- hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is only inactive against *Streptococcus mutans*. All 3-hydroxyflavone derivatives exhibited their MIC to be in range of 250-125 µg/ml., 2,3-dihydroflavan-3-ol derivatives exhibited their MIC to be in range of 1000- 500 µg/ml. The chalcone derivatives exhibited their MIC to be at 250 µg/ml.

Keywords: *Streptococcus mutans,* flavonoids derivatives, MIC, 2,3-dihydroflavan-3-ol.

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INTRODUCTION:

Flavonoids are secondary metabolite. These are synthesized by phenylpropanoid pathway.

Series of enzyme Phenylalanine Ammonia Lyase (PAL), cinnamate 4- hydroxylase (C4H) & 4-Coumarate CoA ligase (4CL) catalyzes the conversion of phenylalanine to 4-coumaroyl-CoA, it then reacts with malonyl CoA. The flavonoid biosynthetic rout is starts with the condensation process of one molecule of 4-coumaroyl- CoA and three molecules of malonyl-CoA, and obtain tetrahydrochalcone. This reaction procedure is catalyzing by the chalcone synthase enzyme(CHS). Chalcone is convert in to a flavanone (naringenin) by the help of enzyme chalcone f- isomerase (CHI). The central naringenin and the intermediate is converted into various side chains and each convert into different groups of flavonoids. Flavanone 3-hydroxylase (F3H) catalyzes the stereo- specific 3-beta-hydroxylation of (2S)-flavanones to dihydro-flavonols (dihydrokampferol).

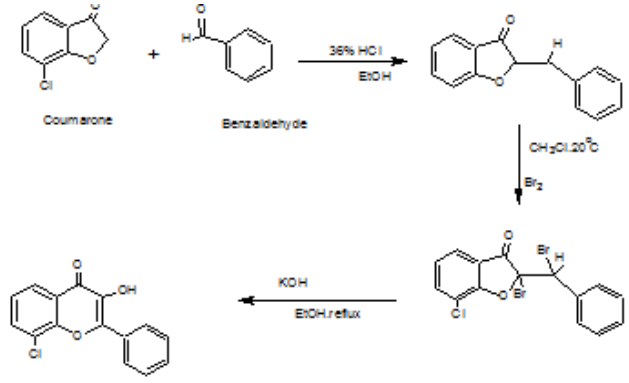
Antimicrobial Activity:

An antimicrobial is a substance that kills or inhibits the growth of microbes such as prokaryotic bacteria, viruses and fungus. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Since the flavonoids are secondary metabolites, they posses inherent ability to combat with infections. Thus they show antimicrobial activity. Lin reported a method of synthesis of flavone containing Beta fluroethyl thiourea and screened for antimycobacterial activity. Sherif B. Abdel Ghani, synthesized flavone derivatives using a modified Baker– Venkataraman rearrangement and evaluated for antimicrobial activity against S.aureus, E.coli etc. Literature states that plant flavonoids in toothpastes are responsible for antiplaque action and works against tooth decay.[1] Plaque-related diseases are probably the most common bacterial diseases occurring in human society. The flavonoid derivatives which posses hydroxylated phenolic nucleus where the C6-C3 unit

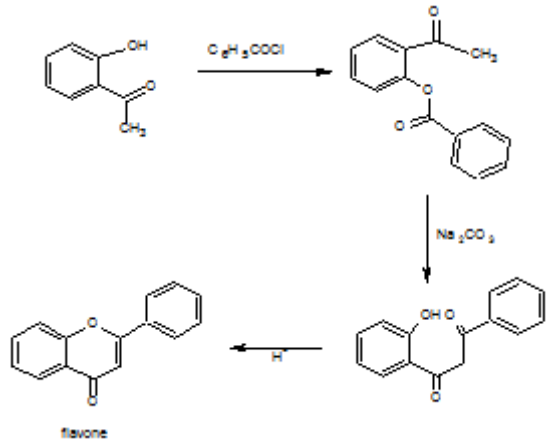
is linked to an aromatic ring, exhibit a broad range of antimicrobial activity. The catechins as found in green tea is the one of example of it.[54] The *streptococcus mutans* is a bacteria that found in the human mouth cavity. This bacterial strain produces plaque and acids that break down tooth enamel and cause dental caries. A gram positive cocci, facultatively anaerobic bacteria that forms rod-like chains.

EXPERIMENTAL WORK:

Synthetic Scheme:



The first step in this mechanism is an acid catalyzed aldol condensation between benzaldehyde and a Coumarone to an ortho-hydroxychalcone. Bromination of the alkene group gives a dibromo-adduct which rearranges to the flavonol by reaction with potassium hydroxide, which yield flavonol.[13,14]



The Baker-Venkataraman rearrangement is the chemical reaction of 2- hydroxyacetophenones with aromatic acylchloride occurs to form 1,3-diketones. This rearrangement reaction proceeds via enolate formation followed by acyl transfer. Then it cyclises into flavone.[13] As the same of above scheme can be worked out as 2-Methoxybenzoyl Chloride is prepared by reaction of 2-methoxybenzoic acid with Thionyl chloride and DMF. 2-Methoxybenzoyl Chloride then added to mixture of 2-hydroxyacetophenone and pyridine, 2-[(2-Methoxybenzoyl) oxy]acetophenone thus obtained is treated with pyridine and KOH which gives1-(2-Hydroxyphenyl)-3-(2- methoxy phenyl)-propan1,3-dione.This is then treated with conc.H2SO4 and acetic acid which gives flavone.

Antibacterial Activity

The synthesize organic compounds were analyse in vitro for their antibacterial activity against *Streptococcus mutans* which is pathogenic in human beings.

Method: the cup plate agar diffusion method using Nutrient agar.

Preparation of Nutrient broth:

Nutrient broth (3.8 gm) was dissolved in 100 ml distilled water and pH was maintained to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 min.

Preparation of Inoculums:

24 hours prior to these testing inoculations of the above bacterial cultures was made in the Nutrient broth and incubated at 37^{0}C for 18-24 hrs.

Preparation of medium:

2.8 gm Nutrient agar was dissolved into 100 ml distilled water It was sterilized by autoclaving at 15 psi for 20 min.

Preparation of test solution:

Each test compound (5 mg) was dissolved in dimethylformamide (5 ml) to give stock solution of concentration 1000 mcg/ml. Then the 0.1 ml of this solution was used for testing.

Method of testing:

Nutrient agar plates were prepared by pouring 15-20 ml of medium into each sterilised petridish and were allowed to set at the room temperature. The cell suspension was inoculated over the surface of agar medium using sterile cotton. The three cups were scooped in each plate using cork borer of 6mm diameter. Then the solution of test compounds (0.10 ml) was added in cups by using micropipettes and these plates are incubated at 37^{0}C for 48 hrs. The zone of inhibition is measured in mm.

Determination of Minimum ihibitory concentration (MIC) Method: Tube dilution /Broth dilution method

Tube dilution technique

One ml of double strength nutrient broth was added to a set of presterilized 5 test tubes (numbered from 1-5). To the first test tube, 1 ml complex solution (conc. =2000 µg/ml) was added. After mixing, a solution (1 ml) from test tube no.1, it was transferred to test tube no.2 so as to obtain concentration of 1000 µg/ml. The same procedure (serial dilution) was followed for the remaining test tubes from no. 3 to no. 5 to get the concentration of compounds in the 500 µg/ml , 250 µg/ml and 125 µg/ml. From the 5th test tube 1 ml of solution was discarded so as to get the equal volume in each test tube. Thus each tube having concentration of (2000, 1000, 500, 250, 125 µg/ml). To each test tube 20 µl of Streptococcus mutans suspension was added (inoculation). All test tubes were incubated at 37˚C for 24 hours and observed the turbidity. This sets of test tube were compared for determining the MIC.

Table 1: MIC experimental set up

Micro-organism	Streptococcus mutans
Media	Double strength nutrient
Conc. of Compounds	100µg/ml
Loaded volume of compounds	1 ml
Loaded Volume of media	1 ml
Volume of microbial suspension load	20 µl
Incubation temperature	37°C
Incubation period	24 Hrs

RUSLTS AND DISCUSSION

Antimicrobial (Antiplaque) Screening

Antimicrobial activity against *Streptococcus mutans* has shown satisfactory results.

Zone of Inhibition

The diameters of ZOI with streptococcus mutans were found to be satisfactory in all derivatives. The observation of ZOI suggested the antibacterial activity of tested compounds J1, J2, J3, J4, J6, V1, V2, V4 V5, R1, R2, R3, R4, R5, R6 against *Streptococcus mutans*.

Minimum inhibitory concentration

On observation of positive results of antimicrobial activity, the MICs of synthesized compounds were determined by tube dilution method. Studies indicated the requirement of inhibitory concentration in order "chalcone derivatives > 2,3-dihydroflavan-3-ol derivatives > 3-hydroxyflavone derivatives. The result of study indicated that C5 [1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is only inactive against *Streptococcus mutans*. All 3-hydroxyflavone derivatives exhibited their MIC to be in range of 250-125

µg/ml., 2,3-dihydroflavan-3-ol derivatives exhibited their MIC to be in range of 1000- 500 µg/ml. The chalcone derivatives exhibited their MIC to be at 250 µg/ml.

Table 2: Zone of inhibition

Compound code	Zone of inhibition (*Streptococcus mutans* 1000µg/ml)
J1	13mm
J2	10 mm
J3	12mm
J4	9mm
J5	NA
J6	15mm
V1	6mm
V2	12mm
V3	7mm
V4	6mm
V5	10mm
V6	NA
R1	10mm
R2	12mm
R3	13mm
R4	10mm
R5	11mm
R6	9mm

Table 3: MIC of flavonoid derivatives with *Streptococcus mutans*

Compound code	% Transmittance			
	1000 µg/ml	**500 µg/ml**	**250 µg/ml**	**125 µg/ml**
J1	37	34	**30**	17
J2	24	26	**29**	16
J3	18	**27**	17	19
J4	33	35	**28**	19
J5	30	31	29	**26**
J6	31	39	**34**	27
V1	30	37	**41**	25
V2	27	33	30	**27**
V3	32	34	**36**	12
V4	33	36	**25**	15
V5	33	35	**28**	19
V6	32	33	30	**28**
R1	30	39	**35**	27
R2	**47**	24	21	25
R3	**34**	15	18	15
R4	58	**43**	23	27
R5	44	**48**	32	30
R6	**53**	16	13	18

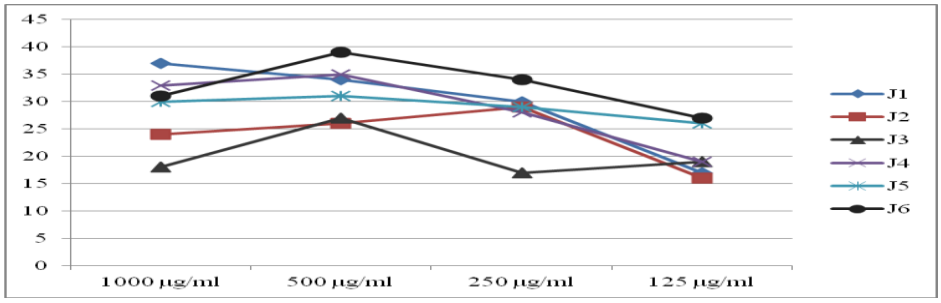


Figure 1 MIC of 2-hydroxy chalcone derivatives

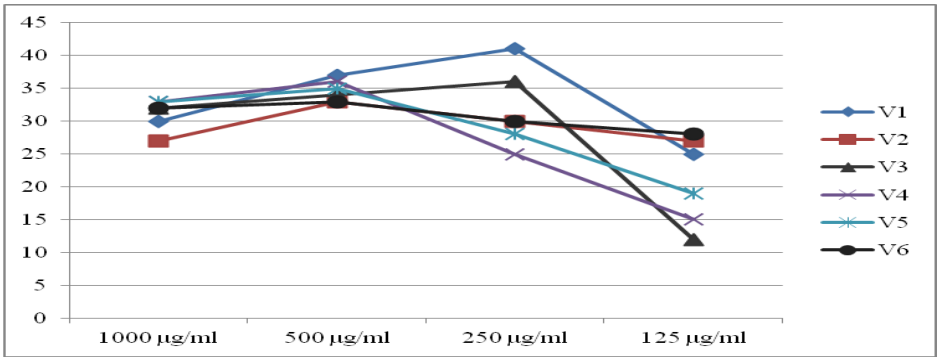


Figure 2 MIC of 3-hydroxy Flavone derivatives

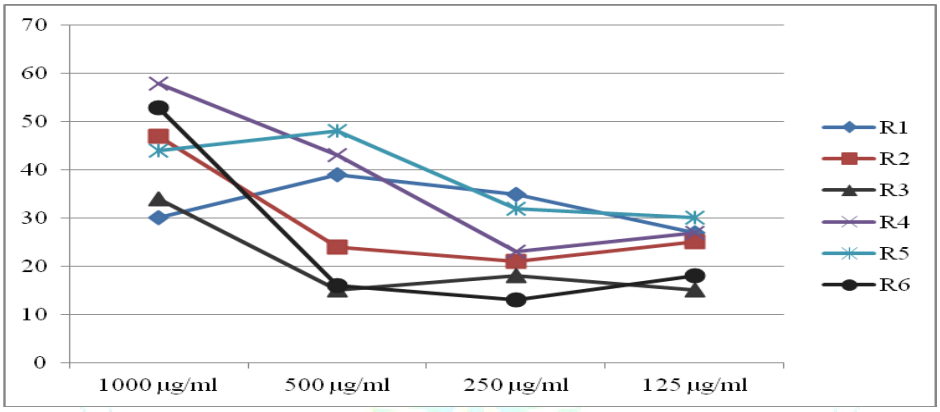


Figure 3 MIC of 2, 3-dihydroxy Flav-3-ol derivatives

The screening results have shown the contribution of electron withdrawing group to antimicrobial activity. As electron withdrawing ability increases

CONCLUSION:

The 2'-hydroxychalcone derivatives, 3-hydroxyflavone derivatives and 2,3- dihydroflavan 3-ol derivatives were synthesized and were confirmed by physicochemical and spectral analysis. The result of study indicated that C5 [1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one]; is only inactive against *Streptococcus mutans*. All 3-hydroxyflavone derivatives exhibited their MIC to be in range of 250-125 µg/ml., 2,3-dihydroflavan-3-ol derivatives exhibited their MIC to be in range of 1000- 500 µg/ml. The chalcone derivatives exhibited their MIC to be at 250 µg/ml.

REFERENCES:

1. Jen Kun Lin and Meng Shih Weng, Flavonoid as Nutraceuticles
2. Halliwell B and Gutteridge J in Free Radicals in Biology and Medicine 2007.
3. Finaud J ; *Sports Medicine* 2006; 35 (4): 327-358
4. Hideo Ohashi, Tetsuya Kyogoku, Takahiro lshikawa, *J Wood Sci* 1999; 45:53-63
5. Haenen GR, Bast A. Nitric oxide radical scavenging of flavonoids. *Methods Enzymol* 1999; 301:490–503.
6. Shuji Kitagawa, Hiromi Sakamoto, and Hiromi Tano, Inhibitory Effects of Flavonoids on Free Radical-Induced Hemolysis andTheir Oxidative Effects on Hemoglobin, *Chem. Pharm. Bull*. 52(8), 2004; 999-1001.
7. Korkina LG, Afanas'ev IB. Antioxidant and chelating properties of flavonoids. *Adv Pharmacol* , 1997; 38:151–63
8. US patent: US 6346364 Zyang .
9. Kerry NL, Abbey M. Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation in vitro. *Atherosclerosis*, 1997; 135:93–102.
10. Shutenko Z, Henry Y, Pinard E, Influence of the antioxidant quercetin in vivo on the level of nitric oxide determined by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. *Biochem Pharmacol* 1999; 57: 199–208.
11. Van Acker SA, Tromp MN, Haenen GR, van der Vijgh WJ, Bast A. Flavonoids as scavengers of nitric oxide radical. *Biochem Biophys Res Commun,* 1995; 214: 755–9.
12. Chang WS, Lee YJ, Lu FJ, Chiang HC., Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Res*, 1993; 13: 2165–70.
13. Manach C, Morand C, Texier O., Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. *J Nutr*, 1995; 125:1911–22.
14. Cos P, Ying L, Calomme M., Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J Nat Prod*, 1998; 61:71–6.
15. Friesenecker B, Tsai AG, Allegra C, Intaglietta M., Oral administration of purified micronized flavonoid fraction suppresses leukocyte adhesion in ischemia-reperfusion injury: in vivo observations in the hamster skin fold. *Int J Microcirc Clin Exp* 1994; 14:50–5.
16. Cho, J.W. Cho, S.Y. Lee, S.R. Lee, K.S. Onion extract and quercetin induce matrix metalloproteinase-1 in vitro and in vivo. Int. J. Mol. Med. 2010; 25, 347–352. [PubMed]
17. Chuang, S.Y. Lin, Y.K.; Lin, C.F. Wang, P.W. Chen, E.L. Fang, J.Y. Elucidating the skin delivery of aglycone and glycoside flavonoids: How the structures affect cutaneous absorption. Nutrients 2017; 9, 1304.
18. Nagoba, B.S. Suryawanshi, N.M. Wadher, B. Selkar, S. Acidic environment and wound healing: A review. Wounds 2015; 27, 5–11. J. Clin. Med. 2020; 9, 109 15 of 17
19. Nagoba, B. Davane, M. Gandhi, R. Wadher, B. Suryawanshi, N. Selkar, S. Treatment of skin and soft tissue infections caused by Pseudomonas aeruginosa—A review of our experiences with citric acid over the past 20 years. Wound Med. 2017, 19:5–9.
20. Bessa, L.J. Fazii, P. Di Giulio, M. Cellini, L. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: Some remarks about wound infection. Int. Wound J. 2013; 12, 47–52.

21. Karpi nski, T.M. Efficacy of octenidine against Pseudomonas aeruginosa strains. Eur. J. Biol. Res. 2019; 9:135–140.
22. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard, 12th ed. CLSI document M02-A12, Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2015 Volume 35, no 1.
23. EUCAST. MIC Determination of Non-Fastidious and Fastidious Organisms. Available online: http://www.eucast. org/ast_of_bacteria/mic_determination (accessed on 26 July 2019).
24. Karpi nski, T.M. Adamczak, A. Fucoxanthin—An antibacterial carotenoid. Antioxidants 2019, 8, 239.
25. Gao, Z. Shao, J. Sun, H. Zhong, W. Zhuang, W.; Zhang, Z. Evaluation of different kinds of organic acids and their antibacterial activity in Japanese Apricot fruits. Afr. J. Agric. Res. 2012; 7:4911–4918.
26. Su, Y. Ma, L. Wen, Y. Wang, H. Zhang, S. Studies of the in vitro antibacterial activities of several polyphenols against clinical isolates of methicillin-resistant Staphylococcus aureus. Molecules 2014; 19:12630–12639.
27. Blaskovich, M.A. Elliott, A.G. Kavanagh, A.M. Ramu, S. Cooper, M.A. In vitro antimicrobial activity of acne drugs against skin-associated bacteria. Sci. Rep. 2019; 9:14658.
28. Basile, A. Giordano, S. López-Sáez, J.A. Cobianchi, R.C. Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry 1999; 52:1479–1482.
29. Afifi, F.U. Abu-Dahab, R. Phytochemical screening and biological activities of Eminium spiculatum (Blume) Kuntze (family Araceae). Nat. Prod. Res. 2012, 26, 878–882.
30. Das, M.C. Sandhu, P. Gupta, P. Rudrapaul, P. De, U.C. Tribedi, P. Akhter, Y. Bhattacharjee, S. Attenuation of Pseudomonas aeruginosa biofilm formation by vitexin: A combinatorial study with azithromycin and gentamicin. Sci. Rep. 2016, 6, 23347.
31. Das, M.C. Das, A. Samaddar, S. Dawarea, A.V. Ghosh, C. Acharjee, S. Sandhu, P.; Jawed, J.J. De Utpal, C. Majumdar, S. et al. Vitexin alters Staphylococcus aureus surface hydrophobicity to interfere with biofilm 2 formation. bioRxiv 2018.
32. Awolola, G.V. Koorbanally, N.A. Chenia, H. Shode, F.O. Baijnath, H. Antibacterial and anti-biofilm activity of flavonoids and triterpenes isolated from the extracts of Ficus sansibarica Warb. subsp. Sansibarica (Moraceae) extracts. Afr. J. Tradit. Complem. Altern. Med. 2014, 11, 124–131.
33. Rammohan, A. Bhaskar, B.V. Venkateswarlu, N. Rao, V.L. Gunasekar, D. Zyryanov, G.V. Isolation of flavonoids from the flowers of Rhynchosia beddomei Baker as prominent antimicrobial agents and molecular docking. Microb. Pathog. 2019, 136, 103667.
34. Aderogba, M.A. Akinkunmi, E.O. Mabusela, W.T. Antioxidant and antimicrobial activities of flavonoid glycosides from Dennettia tripetala G. Baker leaf extract. Nig. J. Nat. Prod. Med. 2011, 15, 49–52.
35. Cottiglia, F. Loy, G. Garau, D. Floris, C. Casu, M. Pompei, R. Bonsignore, L. Antimicrobial evaluation of coumarins and flavonoids from the stems of Daphne gnidium L. Phytomedicine 2001, 8, 302–305.
36. Ali, H. Dixit, S. In vitro antimicrobial activity of flavanoids of Ocimum sanctum with synergistic effect of their combined form. Asian Pac. J. Trop. Dis. 2012, 2, S396–S398.
37. Celiz, G. Daz, M. Audisio, M.C. Antibacterial activity of naringin derivatives against pathogenic strains. J. Appl. Microbiol. 2011, 111, 731–738.
38. Akhtar, M.S. Hossain, M.A. Said, S.A. Isolation and characterization of antimicrobial compound from the stem-bark of the traditionally used medicinal plant Adenium obesum. J. Tradit. Complem. Med. 2017, 7, 296–300.
39. Singh, M. Govindarajan, R. Rawat, A.K.S. Khare, P.B. Antimicrobial flavonoid rutin from Pteris vittata L. against pathogenic gastrointestinal microflora. Am. Fern J. 2008, 98, 98–103.
40. Liu, H. Mou, Y. Zhao, J. Wang, J. Zhou, L. Wang, M. Wang, D. Han, J. Yu, Z. Yang, F. Flavonoids from Halostachys caspica and their antimicrobial and antioxidant activities. Molecules 2010, 15, 7933–7945. [CrossRef] [PubMed] J. Clin. Med. 2020, 9, 109 16 of 17
41. Banerjee, K. Banerjee, S. Das, S. Mandal, M. Probing the potential of apigenin liposomes in enhancing bacterial membrane perturbation and integrity loss. J. Colloid Interface Sci. 2015, 453, 48–59.
42. Ekambaram, S.P. Perumal, S.S. Balakrishnan, A. Marappan, N. Gajendran, S.S. Viswanathan, V. Antibacterial synergy between rosmarinic acid and antibiotics against methicillin-resistant Staphylococcus aureus. J. Intercult. Ethnopharmacol. 2016, 5, 358–363.
43. Smiljkovic, M. Stanisavljevic, D. Stojkovic, D. Petrovic, I. Vicentic, M.J. Popovic, J. Golic Grdadolnik, S. Markovic, D. Sankovic-Babice, S. Glamoclija, J. et al. Apigenin-7-O-glucoside versus apigenin: Insight into the modes of anticandidal and cytotoxic actions. EXCLI J. 2017, 16, 795–807.
44. Matejczyk, M. Swisłocka, R. Golonko, A. Lewandowski, W. Hawrylik, E. Cytotoxic, genotoxic and antimicrobial activity of caffeic and rosmarinic acids and their lithium, sodium and potassium salts as potential anticancer compounds. Adv. Med. Sci. 2018, 63, 14–21.
45. Ren, G. Xue, P. Sun, X. Zhao, G. Determination of the volatile and polyphenol constituents and the antimicrobial, antioxidant, and tyrosinase inhibitory activities of the bioactive compounds from the by-product of Rosa rugosa Thunb. var. plena Regal tea. BMC Complem. Altern. Med. 2018, 18, 307.
46. Huang, C.Y. Inhibition of a putative dihydropyrimidinase from Pseudomonas aeruginosa PAO1 by flavonoids and substrates of cyclic amidohydrolases. PLoS ONE 2015, 10, e0127634.
47. Bustos, P.S. Deza-Ponzio, R. Páez, P.L. Cabrera, J.L. Virgolini, M.B. Ortega, M.G. Flavonoids as protective agents against oxidative stress induced by gentamicin in systemic circulation. Potent protective activity and microbial synergism of luteolin. Food Chem. Toxicol. 2018, 118, 294–302.
48. Xie, Y. Yang, W. Tang, F. Chen, X. Ren, L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr. Med. Chem. 2015, 22, 132–149.
49. Górniak, I. Bartoszewski, R. Króliczewski, J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem. Rev. 2019, 18, 241–272.
50. Ohemeng, K.A. Schwender, C.F. Fu, K.P.; Barrett, J.F. DNA gyrase inhibitory and antibacterial activity of some flavones. Bioorg. Med. Chem. Lett. 1993, 3, 225–230.
51. Lee, J.H. Regmi, S.C. Kim, J.A. Cho, M.H. Yun, H.; Lee, C.S. Lee, J. Apple flavonoid phloretin inhibits Escherichia coli O157:H7 biofilm formation and ameliorates colon inflammation in rats. Infect. Immun. 2011.