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RESEARCH ARTICLE

SYNTHESIS AND STUDY OF ANTIMICROBIAL & ANTI-OXIDANT PROPERTIES OF SUBSTITUTED DERIVATIVES OF COUMARIN.

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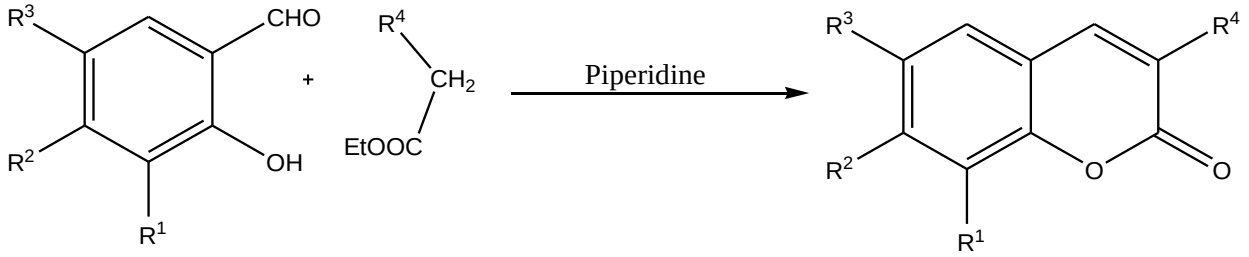
Abstract

Coumarins and their derivatives are very important organic compounds; they are biologically active and widely occur in nature[1,2]Recent studies have been revealed that coumarin and the derivatives exhibit several other medicinal applications such as anti-coagulants, antifungal, insecticidal, hypnoticsphytoalexins, HIV protease & inhibitors[3]. Thus the synthesis of coumarins is of continuing interest. **Potassiumdihydrogen phosphate** a commercially available environmentally benign catalyst non-toxic widely used for the synthesis of the substituted coumarin[4] .The scope of this catalyst has not been fully explored, but can be used as buffer, neutralizing agent. Owing to the numerous advantages associated with cheap and non-hazardous catalyst, and also realizing g the importance of coumarin herein we would like to focus the eco –friendly method for his synthesis of derivatives of coumarin using cheaper and commercially available acid catalysts Potassium dihydrogen phosphate and also by the Knoevenagel condensation under microwave irradiation. The synthesized coumarin derivative was screened in Vitro anti-microbialefficacy testing and anti oxidant properties. In vitro anti-microbial efficacy testing was carried out by broth dilution method by broth dilution method as mentioned in "Pharmaceutical Microbiology". For anti-bacterial activity, MullerHinton medium was used as the nutrient media. Test bacterial species used are Escherichia coli ,(ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungi species used are Aspergilliusniger(ATCC 16404) and Candida albicans (ATCC 10231) in different concentrations starting from 25ppm .All the coumarin derivatives are active against the test bacteria and fungai in different concentrations. Anti-oxidant studies of all these derivatives have been carried out . These compounds were characterized by IR, NMR spectra's. This paper focuses is to develop environmentally reactions, simple, highly efficient and high yielding protocol for the synthesis of coumarin derivatives using Potassiumdihydrogen phosphate as a catalyst. Therefore owing the importance of Potassium dihydrogen phosphate a facile catalyst used for the green synthesis of new derivatives of coumarin.

Experimental Section:-

Condensation of 5-bromo 2-hydroxy benzaldehyde with, ethyl acetoacetate, and ethyl cyanoacetate in the presence of piperidine leads to the synthesis of derivatives of coumarin by a solvent free reaction under microwave irradiation. (Figure-1)

Scheme-1 1

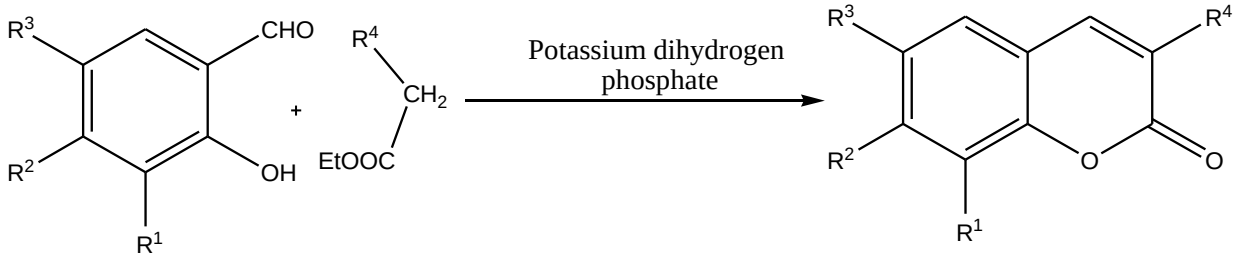


1 2 3f-g

Figure-1: Synthesis of coumarin derivatives by Knoevenagel condensation under microwave irradiation

Condensation of 5-bromo 2-hydroxy benzaldehyde with dimethyl malonate, in the presence of Potassium dihydrogenphosphate catalyst leads to the synthesis of derivatives of coumarins.(Figure-2)

Scheme-2 2



1 2 3f-g

Figure-2Synthesis of derivatives of Coumarin using Potassium dihydrogen phosphate as catalyst.(Refer Table A,B&C)

Table -A

Sr.No	Compound Code No.	R^1	R^2	R^3	R^4
1	3f	H	H	Br	COMe
2	3g	H	H	Br	CN

Table-B

Sr.No.	Compound code No.	Compounds name	Melting point ^oC	Yield %
1	3f	3-acetyl-6-bromo -1-chromen-2-one	172	108
2	3g	6-bromo1-chromen-2-one3-carbonitrile	208	70

Table -C

Sr.No	Compoud Code No.	IR(KBr) ύ cm^{-1}	^{1}HNMR: δ (ppm)
1	3f	650,1350,1200, 1720, 3070,1590	2.48(s,3H),8.50(s,1H),(7.50)s,1H), 7.65(d,1H),7.78(d,1H)
2	3g	600,1230,3070, 1570,1200,1720	8.48(s,1H),7.28(s,1H),7.72(d,1H), 7.65(d,1H)

Anti-microbial studies:-

Pharmacology analysis:-

In Vitro Antibacterial Assay:-

Anti bacterial testing is carried out at Haffkine Institute for Training Research & Testing.

The anti-microbial activity of newly syntheisiedCoumarins was conducted against Escherichia coli ,(ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungi species used are Aspergillius Niger(ATCC 16404) and Candida albicans (ATCC 10231) .Ampicillin for anti-bacterial test and fluconazole for anti fungal test was employed as reference to compare t

Results:-

Nutrient broth was used for the preparation of inoculation of the bacteria and nutrient agar was used for the screening methods.

The synthesized new coumarinderivatives(heterocyclic compounds) were screened in Vitro anti-microbial efficacy testing. In vitro anti-microbial efficacy testing was carried out by broth dilution method as mentioned in "Pharmaceutical Microbiology"Edited by W.BHugo&A.D.Russel,Sixth Edition ,Blackwell Science publication . The concentration of the samples used were 25 ppm 50ppm,100ppm,150ppm&200ppm. Initially, Dimethyl sulphoxide solvent was used to prepare stock solution of 1000ppm.of all samples separately; then further required dilutions were done in respective broth medium i.e Muller Hinton medium. For anti-bacterial activity, Muller Hinton broth was used as the nutrient media. Test bacterial species used are Escherichia coli ,(ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungi species used are Aspergillius Niger(ATCC 16404) and Candida albicans (ATCC 10231) in different concentrations.The four different concentrations of the samples 25 ppm ,50 ppm ,100 ppm ,150 ppm &200 ppm were prepared and taken in Muller Hinton broth separately in sterile test tube and to each individual test tube 0.1 cm^3 of above mentioned bacterial suspension was added (having approximately1.0 x 106 *CFU). These tubes were then kept for incubation at 37^oC for 48 hours. To check the growth if any.

***CFU = Colony formin**

**** N = No growth or bacteria was killed / inactivated**

MIC= minimum inhibitory concentration expressed in ppm (parts per million)

Compound 3f Table-D:-

Sr.No	Test bacterial species	Standard reference sample	Inhibition\ Viability of the test bacterial species after 48 hours of incubation in the concentration of			
		Ampicillin/fluconazole (MIC) (ppm)	25ppm	50ppm	100ppm	150ppm
1	Pseudomonas Aeruginosa (Fisher's immunotype-IV)	150	V	V	**N	N
2	Escherichia coli (ATCC 10148),	100	V	V	N	N
3	Staphylococcus aureus(NCTC 3750),	100	V	V	N	N
4	Aspergillius Niger(ATCC 16404)	150	V	V	N	N
5	Candida albicans (ATCC 10231)	100	V	V	N	N

***CFU = Colony formin**

**** N = No growth or bacteria was killed / inactivated**

MIC= minimum inhibitory concentration expressed in ppm (parts per million)Compound labelled as' 3f,' kills /inactivates the test organism Escherichia coli,(ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungai species used are Aspergillius Niger(ATCC 16404) and Candida albicans (ATCC 10231 in the concentration of 100 ppm, In other words the compound 3f has shown the anti-

bacterial/antifungal activities in the concentration of 100 ppm, against the above mentioned test bacterial/fungal species . whereas Standard reference sample ampicillin/fluconazole (MIC) at 100ppm in the same condition against Escherichia coli ,(ATCC 10148), and Staphylococcus aureus(NCTC 3750),but against Pseudomonas aeruginosa is150ppm. Standard reference sample fluconazole shows MIC at 100ppm against Candida albicans (ATCC 10231) but 150 ppm against Aspergillius Niger (ATCC 16404)

Compound 3gTable-E:-

Sr.No	Test bacterial species	Standard reference sample	Inhibition\ Viability of the test bacterial species after 48 hours of incubation in the concentration of			
		Ampicillin/fluconazole (MIC) (ppm)	25ppm	50ppm	100ppm	150ppm
1	Pseudomonas Aeruginosa (Fisher's immunotype-IV)	150	V	V	**N	N
2	Escherichia coli ,(ATCC 10148),	100	V	V	N	N
3	Staphylococcus aureus(NCTC 3750),	100	V	V	N	N
4	Aspergillius Niger(ATCC 16404)	150	V	V	N	N
5	Candida albicans (ATCC 10231)	100	V	V	N	N

***CFU = Colony formin**
**** N = No growth or bacteria was killed / inactivated**
MIC= minimum inhibitory concentration expressed in ppm (parts per million)

Compound labelled as' 3g,' kills /inactivates the test organism Escherichia coli(ATCC 10148), Staphylococcus aureus(NCTC 3750), Pseudomonas aeruginosa (Fisher'Immunotype IV), test fungai species used are Aspergillius Niger(ATCC 16404) and Candida albicans (ATCC 10231 in the concentration of 100 ppm, In other words the compound 3g has shown the anti-bacterial/antifungal activities in the concentration of 100 ppm, against the above mentioned test bacterial/fungal species . Whereas Standard reference sample ampicillin/fluconazole (MIC) at 100ppm in the same condition against Escherichia coli ,(ATCC 10148), and Staphylococcus aureus(NCTC 3750),but but 150 ppm against Aspergillius Niger (ATCC 16404)against Pseudomonas aeruginosa at 150ppm. Standard reference sample fluconazole shows MIC at 100ppm against Candida albicans (ATCC 10231)

Anti-oxidant activity:-

The 1,1-diphenyl-2-picrylhydrazyl radical has been widely used to evaluate the free radical scavenging capacity of different antioxidants[5-7]Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidativestress.DPPH Radical Scavenging Activity 10 ml of the different concentrations of samples /standard was centrifuged at 3000 rpm using a centrifuge for 10 minutes and collected. The supernatant of the extract (1 ml) was added to 3 ml of methanolic solution of ofof DPPH (20 mg/l) in a test tube. The reaction mixture was kept at 250C for one hour in an incubator .The absorbance of the residual(1,1-Diphenyl-2-picrylhydrazy)l DPPH solution was determined at 517 nm in a UV-Visible Spectrophotometer. The experiment was performed in triplicate. The standard used was BHT ButyratedHydroxy Toluene as positive control .The inhibition was calculated in following formula,I (%) = 100 x $(A_0-A_1)/A_0$Where A_0 is the absorbance of the control; A_1 is the absorbance the extract/standard, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for % 50 inhibition was determined and expressed as IC50 value. The bromo substituted derivatives ofcoumarin found to be better antioxidant capacity.

The result is as shown **Table-F:-**

Antioxidant activity			
Sr.No	standard	Sample Code	IC_{50}+SD
1	BHT(ButyratedHydroxyToulene)	BHT	8.25+0.336
2		3f	46.00+ 1.77
3		3g	34 + 1.36

Conclusion:

Mild reaction conditions, short reaction time, simple experimental work up cheapness of the reagents are the noteworthy advantages of this environment friendly protocol.

All the synthesized compounds are found to possess good anti-bacterial/anti-fungal activity when compared with the standard. Even some compounds are showing greater anti-bacterial /antifungal activities compared to the standard reference sample ampicillin/fluconazole.They are found to be better antioxidants too.

References:-

1. Kennedy R. D; and Thomas R. D; John willey and sons, Chichester 1997.
2. Murray, R.D.H.; Mendez J. B. S.A; Wiley and Sons: New York, 1982.
3. (a) Cravotto,G. ; Palmisano G.M. ; Tagliapietra, G. S; Tetrahedron: Asymmetry 12,
4. (707-709) 2001.
5. (b) Wang C.J; Hsieh, Y.J; Chu, C.Y; Lin,Y.L;. Tseng, T.H ; Cancer Lett. 183, (163-168) 2002.
6. (c) Sardari S; Nishibe S; Daneshtalab M. ; Stud. Nat. Prod. Chem. 23, (335-393) 2000.
7. Dabiri Ma; Bashiribod S ;Molecules 14 (1126 – 1133) 2009.
8. 5). Brand-Willams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft and Technologie.1995; 28: 25-30.
9. 6). Espin JC, Soler-Rivas C, Wichers HJ. Characterization of total free radical scavenger capacity of vegetable oils and oil fraction using 2,2- diphenyl-1-picrylhydrazyl radical. J Agric Food Chem. 2000; 48:
10. 648-656.
11. 7) Yu L. Free radical scavenging properties of conjugated linoleic acids.J Agric Food Chem. 2001; 49: 3452-56.