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Effects Of Coumarin And Its Derivatives On The Expression Of Exopolysaccharide glucosyl transferase Gene epsG In Streptococcus Pneumoniae P3.

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ABSTRACT

Estimation of effects of coumarin,7-ethyl-4-methyl coumarin, 4,7dimethyl-6-nitro coumarin and7-hydroxy-4-methyl coumarin on the expression fold of eps Ggene with different concentration (100 ,200, 300 and 400)µg/ml. The results shown that the Coumarin and its derivatives at the concentration 200 µg/ml was more effect in expression fold epsG gene compared with control. The gene expression of epsG gene responsible of production of glucosyl transferase affected by coumarin and its derivatives at different ratio.

Keywords: Coumarin, Glucosyltransferase, epsG gene

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INTRODUCTION

Glycosyltransferases (GTs) are a large family of enzymes that are essential in all domains of life for the biosynthesis of complex carbohydrates and glycoconjugates. GTs catalyse the transfer of a sugar from a glycosyl donor to a variety of acceptor molecules, for example, oligosaccharides, peptides, lipids or small molecules. Such glycosylation reactions are central to many fundamental biological processes, including cellular adhesion, cell signalling and bacterial- and plant-cell-wall biosynthesis. GTs are therefore of significant interest as molecular targets in chemical biology and drug discovery (1).Therefore, the change of glycosyltransferase expression and activity are relevant to many diseases and can serve as a diagnostic marker for certain diseases. For examples, α 1,3-galactosyltransferase activity in the body can lead to autoimmune response or organ allograft rejection (2).

Coumarins displaying activities such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, anti-hypertensive, antitubercular, , Cytochrome P450 inhibiting, anti-hyperglycemic, antioxidant, and neuroprotective. coumarins are a family of benzopyrones (1,2-benzopyrones or 2H-1-benzopyran-2-ones) are widely distributed in the nature. They represent an important family of naturally occurring and/or synthetic oxygen-containing heterocycles, bearing a typical benzopyroneframework (3).

Also industrial additives such as perfumes and cosmetics.coumarin compounds such as medicinal candidates for drugs with strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, broad spectrum, and better curative effects.(4 ; 5).

Capsule is essential for pneumococcal virulence and provides a barrier against recognition by phagocytic receptors of complement bound to the bacterial surface(6). In addition,the capsule can affect the amount of complement that binds to the bacterial surface (7),and it is required for the asymptomatic colonization of the nasopharynx (8**).**

Bender et al., (2003) (9)the enzymes uniquely required for capsule synthesis in S. pneumoniae are encoded within a single locus on the chromosome . The genes are organized in a Cassette structure with type-specific genes needed to synthesize each capsule being flanked by genes that are common to all capsule types .CpsA, -B, -C, and -D, encoded by the upstream common genes, are highly conserved among serotypes and have been proposed to play a role in modulating capsule amounts and chain length. CpsE, the initiating glycosyltransferase, is encoded by the next gene in most S. pneumoniae capsule loci and has been designated a type-specific gene . However, it also is highly conserved among serotypes, with the majority of CpsE proteins sharing 70 to 98% amino acid identity (10**).**

The capsule locus of S. pneumoniae encodes all the enzymes required for assembling the repeat unit Fig.(1). For all Wzy-dependent serotypes, this genetic region is organized in a similar cassette-like arrangement.S.pneumoniae, the Wzy-dependent loci lie between dexB and aliA in the chromosome . The 5` region of the locus contains four conserved sequences that are important in the modulation of capsule synthesis and, in most serotypes, have the order cpsABCD (wzg, wzh, wzd, wze). The next gene encodes the initiating glycosyltransferase. In the 68 serotypes where synthesis initiates with Glc(glucose),thissequence encodes a CpsE homolog(WchA homology group; poly isoprenyl phosphate hexose-1-P family). The remaining serotypes lack Glc, and initiation with another sugar is catalyzed by a glycosyltransferase of the WciI, WcjG, or WcjH homology group (11). Among all serotypes, the cpsA sequences are highly conserved, whereas the cpsBCD sequences (and cpsE, where present) can be divided into two clusters The downstream regions of the loci do not exhibit the sequence conservation or clustering seen in the upstream regions, and they are considered to be serotype specific. These regions encode the enzymes responsible for polymer-specific functions, such as the synthesis of NDP-sugars unique to the capsule structure, polymerization (Wzy polymerase), transport (Wzxflippase), glycosidic linkages (glycosyltransferases), and sugar modification (O-acetylases).Differences in structure that result in cross-reactive but distinct serotypes can be due to the activity of the Wzy polymerase (as noted above), modification enzymes that affect O-acetylation of the repeat unit ,or replacement of one glycosyltransferase with another (12)**.** The only known active promoter for Wzy loci in S. pneumoniae lies upstream of cpsA (Figure 1), and the genes appear to be transcribed as a single operon as is the case in other bacteria

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Individual ribosome binding sites are present for each gene, and often occur in the 3` region of the preceding gene; thus, translational control may be important. CpsA has been proposed to be a transcriptional regulator because of its homology with LytR in Bacillus subtilis, and cpsA deletion mutants of S.pneumoniae and S. agalactiae do produce less capsule . It is only in S. agalactiae, however, that significant reductions in transcription of the cps genes resulting from cpsA mutation have been reported (13; 14).

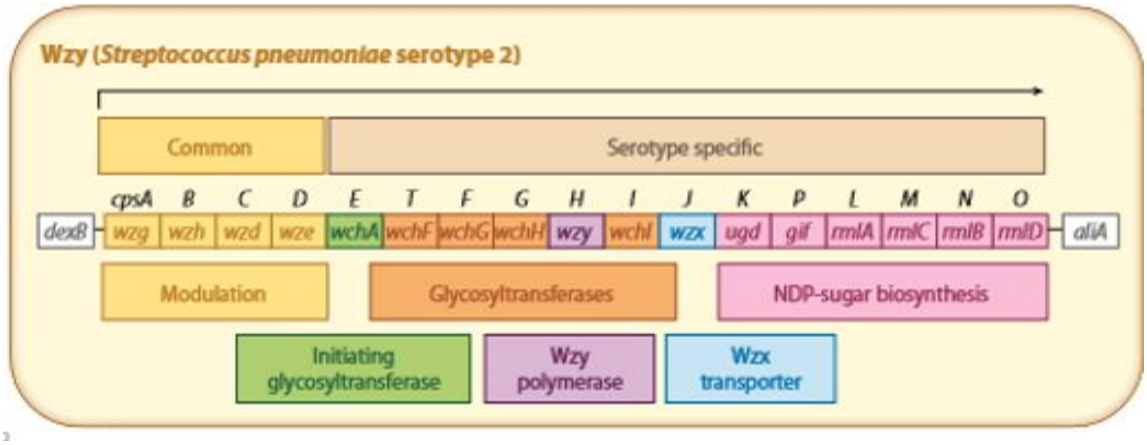

Figure (1):S. pneumoniae, the Wzy-dependent loci between dexB and aliA in the chromosome (Yother ,2011) (14).

MATERIALS AND METHODS

Preparation of coumarin and its derivatives:

The coumarin and its derivatives were prepared by the Chemistry dept. Al-Mustansiriya University by Prof. Dr. Redha I. Al-Bayati and his team. It was prepared by dissolved 1000µg of each compound and dissolved in 1 ml DMSO , from this stock prepared different concentration (100 ,200,300 and 400) µg/ml.

Total RNA extraction

This took place after growing the S.pneumoniae isolate in todd-hewitt broth +0.5% yeast extract (without coumarin and its derivatives) and incubated overnight at 37°C in candle jar, and the experiment with inhibitors S.pneumoniae was cultured in todd-hewitt broth+0.5% yeast extract for 3 hr at 37°C in candle jar ,then add the coumarin and its derivatives and re-incubated overnight at 37°C in candle jar ,to detect extract RNAaccording to DSBIO Kit.aftre that convert the RNA to cDNA synthesis for mRNA

Total RNA was reversely transcribed to complementary DNA (cDNA) using WizScript™ RT FDmix Kit. The procedure was carried out in a reaction volume of 20 µl according to the manufacturer's instructions. The total RNA volume to be reversely transcribed was (20µl).

Quantitative Real Time PCR (qRT–PCR)

The expression levels of exopoly saccharids glucosyltransferaseepsGgene was estimated by qRT-PCR. To confirm the expression of target gene, quantitative real time qRT-PCR SYBR Green assay was used. Primers sequences for epsG gene was prepared according to (synthesized by Alpha DNA Ltd (Canada) and stored lyophilized at (-23°C).
The mRNA levels of endogenous control gene **glucose kinase gkihousekeeping**were amplified and used to normalize the mRNA levels of the epsG genes.

Protocol:

1-All reagents were thawed on ice, and each solution was gently mixed.
2- Take sterilized Eppendorf, then prepared the component of reaction according table (1).

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Table (1): component of reactionQuantitative Real Time PCR

Component	Volume(µl)\Reaction
Mastermix	**10**
forward primer	**1**
reverse primer	**1**
cDNA	**2**
Nuclease-free H2O	**6**

3-Eppendorfswere placed in a thermal cycler program as in table (2)

Table (2):Thermal profile of epsG,gkigene expression

Initial denaturation	**95ºC**	**5 min**	**Hold**
Denature	**95ºC**	**20 sec**	**40**
Anneal/extend	**60ºC**	**30 sec**	
Dissociation			

RESULTS

Effect different concentration of coumarin on epsG expression

The fold of gene expression revealed the concentration 200 µg of coumarin was more effects and low the expression to **0.3898 ± 0.02** compared with control .while the other concentration appeared a slight effects these present in table (3) and figure (2).

Table 3: Fold of Targit gene expression. Depending on $2^{-\Delta\Delta Ct}$Method/ 24hrof inhibitor 1 in different concentration

Con. Inhibitor 1 µg/ml	Means Ct of Targit gene	Means Ct of H.K.	ΔΔCt (Means ΔCt ofTargit gene - Means ΔCt of Control.)	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	Fold of gene expression
100	25.42	12.17	-1.81	3.506	0.0001026/ 3.63	0.9661±0.05 AB
200	26.73	12.17	-0.5	1.41	1.41/ 3.63	0.3898 ± 0.02 C
300	25.80	12.17	-1.43	2.69	2.69/ 3.63	0.7419 ± 0.05 B
400	25.52	12.17	-1.71	3.27	3.27/ 3.63	0.9011±0.06 AB
Untreated	25.37	12.17	-1.86	3.63	3.63/ 3.63	1.00 ± 0.02 A
LSD value	---	---	---	---	---	0.214 **

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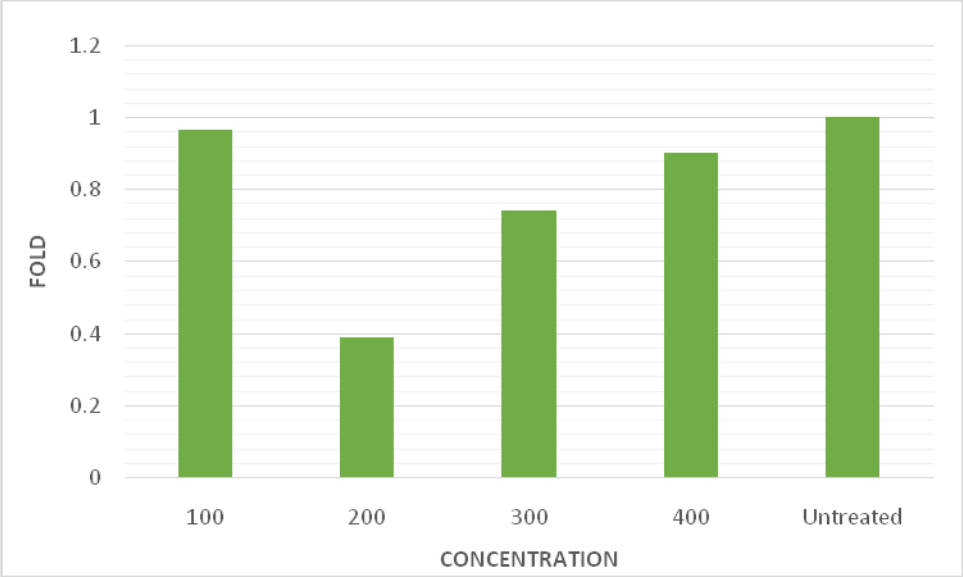

Figure (2): Effect different concentration of coumarin on epsG expression

Effect different concentration of 7-ethyl-4-methyl coumarin on epsG expression:

Table (4) clarified that differences among concentration of inhibitor were significant ($p<0.01$).the highest mean for gene expression was detected in concentration 100 µg (**0.965 ± 0.07).**whereas in concentration 200 µg was best effect that decrease gene expression to (**0.36 ± 0.02),** comparing with control **(1.00 ± 0.02).**

Table (4): Fold of Targit gene expression. Depending on $2^{-\Delta\Delta Ct}$Method/ 24hr of inhibitor 2

Con. Of inhibitor 2 µg/ml	Means Ct of Targit gene	Means Ct of H.K.	ΔΔCt (Means ΔCt ofTargit gene - Means ΔCt of control)	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	Fold of gene expression
100	22.42	16.60	-9.24	604.66	604.66/625.99	0.965 ± 0.07 A
200	23.83	16.60	-7.83	227.54	227.54/625.99	0.36 ± 0.02 C
300	23.07	16.60	-8.59	385.34	385.34/625.99	0.61 ± 0.05 B
400	22.77	16.60	-8.89	474.41	474.41/625.99	0.75 ± 0.05 AB
Untreate	22.37	16.60	-9.29	625.99	625.99/625.99	1.00 ± 0.02 A
LSD value	---	---	---	---	---	0.255 **

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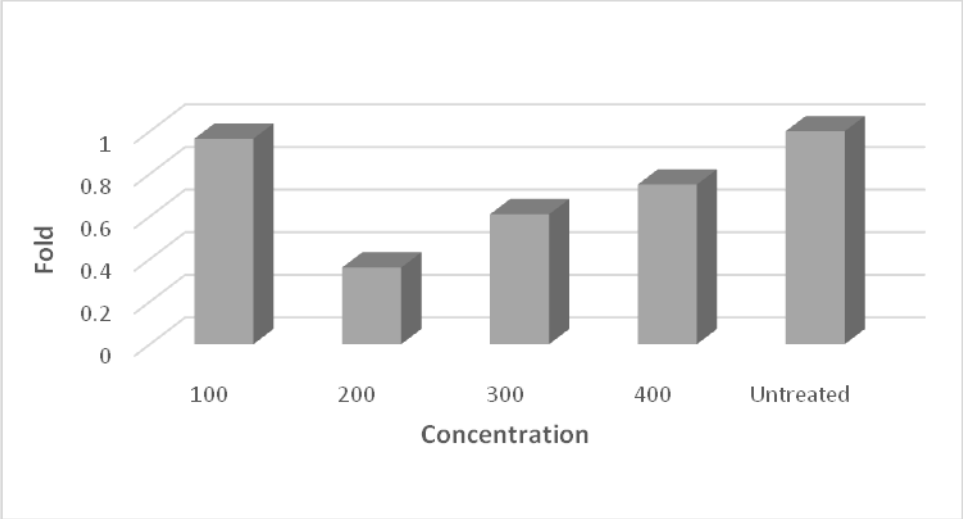

Figure3: Effect different concentration of 7-ethyl-4-methyl coumarin on epsG expression

Effect different concentration of 4,7-dimethyl-6-nitrocoumarin on epsG expression

The compound **4,7-dimethyl-6-nitrocoumarin** used in different concentration illustrate in the table (5) appeared highest value in fold of gene expression in concentration 100 µg was **(1.079 ± 0.09),** comparing with control (**1.00 ± 0.02**) with LSD value **0.184.**while the concentration 200 µg (**0.1767 ± 0.01)** more effects on the expression of gene.

Table 5: Fold of Targit gene expression. Depending on$2^{-\Delta\Delta Ct}$Method/ 24hr of inhibitor 3 in different con

Con.of inhibitor 3 µg/ml	Means Ct of Targit gene	Means Ct of H.K.	ΔΔCt (Means ΔCt ofTargit gene - Means ΔCt of H.K.)	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	Fold of gene expression
100	21.95	11.75	-4.86	29.04	29.04/26.90	1.079 ± 0.09 A
200	24.56	11.75	-2.25	4.75	4.75/26.90	0.1767 ± 0.01 C
300	22.98	11.75	-3.83	14.22	14.22/26.90	0.5281 ± 0.06 B
400	22.73	11.75	-4.08	16.91	16.91/26.90	0.6284 ± 0.06 B
Untreated Control	22.05	11.75	-4.75	26.90	26.90/26.90	1.00 ± 0.02 A
LSD value	---	---	---	---	---	0.184 **

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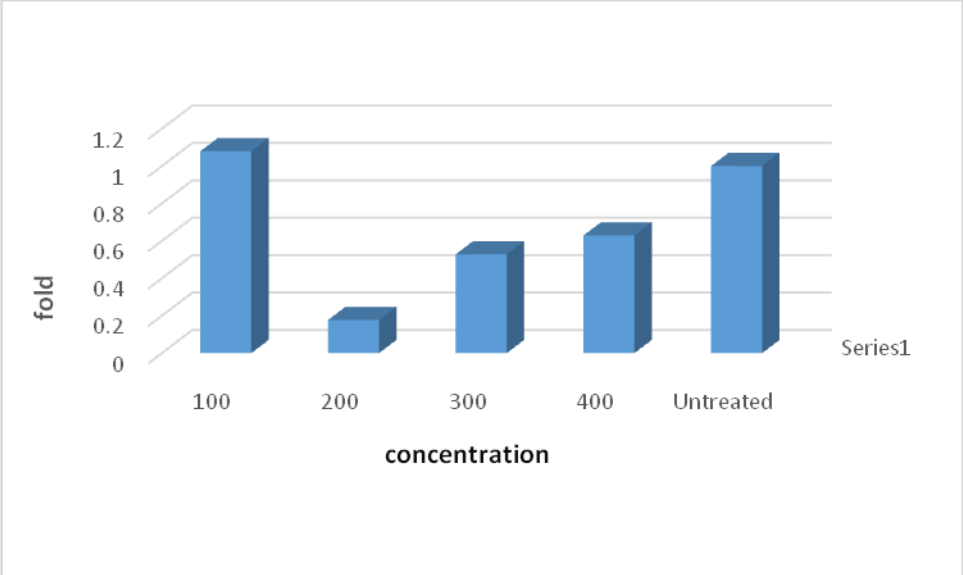

Figure (4): Effect different concentration of 4,7-dimethyl-6-nitrocoumarin on epsG expression

Effect different concentration of 7-hydroxy-4-methyl coumarin on epsG expression:

The structure of **7-hydroxy-4-methyl coumarin** have the ability to antibacterial due to adding hydroxyl group. The concentration 200µg was (**0.4891 ± 0.03**) more effective. Whereas other concentration 100,300,400 µg decrease the expression of gene near to the control appeared in table (6) .

Table (6): Fold of Targit gene expression. Depending on $2^{-\Delta\Delta Ct}$ Method/ 24hr of inhibitor 4 in different concentration.

Con. of inhibitor 4 µg/ml	Means Ct of Targit gene	Means Ct of H.K.	ΔCt (Means ΔCt ofTargit gene - Means ΔCt of H.K.)	$2^{-\Delta\Delta Ct}$	experimental group/ Control group	Fold of gene expression
100	27.41	20.27	-7.92	242.19	242.19/333.14	0.7269 ± 0.06 B
200	27.98	20.27	-7.35	163.14	163.14/333.14	0.4891 ± 0.03 C
300	27.60	20.27	-7.73	212.30	212.30/333.14	0.6373±0.06 BC
400	27.70	20.27	-7.63	198.08	198.08/333.14	0.5926±0.04 BC
Untreated Control	26.95	20.27	-8.38	333.14	333.14/333.14	1.00 ± 0.02 A
LSD value	---	---	---	---	---	0.216 **

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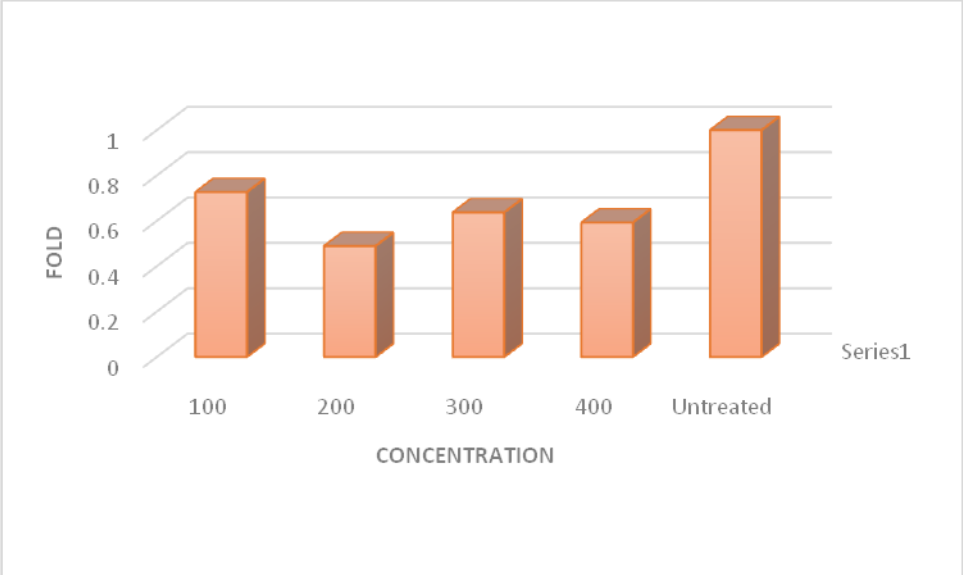

Figure(5):**Effect different concentration of 7-hydroxy-4-methyl coumarin on epsG expression**

DISCUSSION

The results agree with (tanitameet al.,2004) mentioned the pyrazole ring affect on DNA gyrase of Staphylococcus aureaus and Streptococcus pneumoniae The coumarinaffect on DNA gyrase and topoisomerase IV The coumarin compounds recently identified as protease and integrase inhibitors.

Coumarins generally possess moderate antibacterial activity and their activities strongly depend on the nature and position of the substituents the antimicrobial activity this might be enhanced by introduction of the ester or carboxylic acid group in the coumarin molecule (15)**.**

Coumarin derivatives are family of proteins with anti-inflammatory, anti-viral, anti-microbial, and antioxidant effects (16)**.** coumarin antibiotics inhibited the ATP that have multi-functions during DNA replication; each is comprised of two subunits: GyrA and GyrB for DNA gyrase and ParC/GrlA and ParE/GrlB for topoisomerase IV. The GyrA and ParC/GrlA proteins contain the DNA binding functions and are targeted by the fluoroquinolones (which are purely synthetic antibiotics), whereas GyrB and ParE/GrlB perform the roles of ATP binding and hydrolysis inhibit by coumarins. (17).

Singh et al.,2017(16) **.**clarified the 7-hydroxy-4-methylcoumarin derivatives possess diverse type of biological activity including antimicrobial and anti-inflammatory activity. The antibacterial activity were shown that compounds exhibited mild-to-moderate antibacterial activity against (Gram-positive) B. subtilis and (Gram negative) E. coli at concentration of 100 µg/ml by disc diffusion. The compounds appeared the antimicrobial activity are against **Bacillus subtilis** , **Staphylococcus aureus** ,**E coli** and **Pseudomonas aeruginosa**show size of zone of inhibition of bacterial growth procedure by test compounds for broad range of antimicrobial activity inhibiting growth (18)..

CONCLUSION

The gene expression of epsG gene responsible of production of glucoyltranferase affected by coumarin and its derivatives at different ratio and this effects on fold of expression gene was significant between that's.

REFERENCES

[1] Wagner, G.K. ; Pesnot, T. (2010)**.**Glycosyltransferases and their assays.Chembiochem. 24: 11(14):1939-1949.

[2] Bao, X. F.; Kobayashi, M.; Hatakeyama, S.; Angata, K.; Gullberg, D.; Nakayama, J.; Fukuda, M. N.; Fukuda, M. (2009). Tumor suppressor function of laminin-binding α dystroglycan requires a distinct β3-N-acetyltglucosaminyltransferase .PNAS, 106, 12109-12114.

[3] Venugopala, K.N.; Rashmi, V.;Odhav, B. (2013).Review on Natural CoumarinLeadCompounds for Their Pharmacological Activity.BioMed Research International.;2013:1-14.

[4] Matos, M.J.; Vina, D.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L. (2012). Focusing onnew monoamine oxidase inhibitors: differently substituted coumarins as an interesting scaffold. Current Topics in Medicinal Chemistry (Sharjah, United Arab Emirates) .12(20):2210-39.

[5] Bubols, G.B.; Vianna, D.R.; Medina-Remon, A. ;von Poser G, Lamuela-Raventos RM, Eifler-Lima VL, et al. (2013).The antioxidant activity of coumarins and flavonoids. Mini-Reviews in Medicinal Chemistry.;13(3):318-34.

[6] Hardy, G. G.; Magee, A. D.; Ventura, C. L.; Caimano, M. J.;Yother, J. (2001).Essential role for cellular phosphor glucomutase in virulence of type 3 Streptococcus pneumoniae. Infect. Immun. 69:2309–2317.

[7] Abeyta, M.; Hardy, G. G.;Yother, J. (2003). Genetic alteration of capsule type but not PspA type affects accessibility of surface-bound complement and surface antigens of Streptococcus pneumoniae. Infect. Immun. 71:218– 225.

[8] Magee, A. D.; J. Yother. (2001). Requirement for capsule in colonization by Streptococcus pneumoniae. Infect. Immun. 69:3755–3761.

[9] Bender, M. H.; Cartee, R. T.;Yother, J. (2003).Positive correlation between tyrosine phosphorylation of CpsD and capsular polysaccharide production in Streptococcus pneumoniae. J. Bacteriol. 185:6057–6066.

[10] van Selm, S.; Kolkman, M. A. B.; van der Zeijst, B. A. M.; Zwaagstra, K. A.; Gaastra, W.; van Putten, J. P. M. (2002). Organization and characterization of the capsule biosynthesis locus of Streptococcus pneumoniae serotype 9V. Microbiology 148:1747–1755.

[11] Bentley, S.D.; Aanensen, D.M. ;Mavroidi, A.; Saunders, D.; Rabbinowitsch, E. et al. (2006). Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. PLoS Genet. 2:e31.

[12] Bratcher, P.E.; Kim, K.H.; Kang, J.H.; Hong, J.Y.; Nahm, M.H. (2010). Identification of natural pneumococcal isolates expressing serotype 6D by genetic, biochemical and serological characterization. Microbiology 156:555–60.

[13] Calix, J. J.;Nahm, M. H. (2010).A new pneumococcal serotype, 11E, has a variably inactivated wcjE gene. J Infect Dis 202:29-38.

[14] Yother, J. (2011). Capsules of Streptococcus pneumoniae and other bacteria: paradigms for polysaccharide biosynthesis and regulation. Annual Review of Microbiology201165:1, 563-581.

[15] Kawasea, M.; Varub, B.; Shahb, A.; Motohashic, N.; Tania, S.; Saito S. et al., (2001).Antimicrobial Activity of New Coumarin Derivatives.Arzneim.-Forsch./Drug Res. 51 (I), 67−71.

[16] Singh, A. D. ; Kumar S.; Saini ; Prabha, H.(2017). Synthesis and biological evaluation some new of 7-hydroxy-4-methylcoumarin derivative.Asian Pacific Journal of Health Sciences. Vol. 4 : Issue 4.

[17] Parmar, K. A.; Patel C. R.; Joshi, S. A.; Patel, R. I.(2013). Synthesis, characterization and antibacterial activity of 2-(4-methyl -7hydroxy-coumarin)-4-(cyclohexylamino)-6-(arylamino)-s-triazine. Der Pharmacia Lettre, 5 (1):24-27.