

Virtual screening to identify protein targets of *Aggregatibacter actinomycetemcomitans* interacting with emodin

ABSTRACT

Aim: The aim of the study is to identify protein targets of *aggregatibacter actinomycetemcomitans* interacting with emodin.

Introduction: *Aggregatibacter mycetemcomitans* is a gram negative bacteria that is associated with localized chronic periodontitis and other systemic diseases. The organism produces a number of virulence factors which provides some benefits to the bacterium. In this study, protein targets of *Aggregatibacter actinomycetemcomitans* interacting with emodin were identified.

Methodology: The present study follows an observational study design wherein we employed computational tools used to identify the targets, assess its functional role and virulence property. The protein targets in the bacteria were identified by virtual screening by using emodin as the compound.

Results: Peptide epitopes present in the virulence factors were identified using the BepiPred tool. The subcellular location of the protein targets were elucidated using emodin as the phytocompound. The 3 - Deoxy - D - manno octulosonic acid, ArcB, hypothetical protein and Arabinose - 5 - phosphate isomerase were found to be virulent.

Conclusion: Within the limits of this study, it provides substantial evidence on the protein targets acting against emodin.

Keywords: *Aggregatibacter*, *emodin*, *novel method*, *periodontitis*, *protein targets*

1. INTRODUCTION:

Oral cavity hosts an abundant collection of microorganisms known to be associated with diseases like periodontitis, dental caries and also deep-seated infections. It is considered to be a perplexing task to eliminate such pathogens from the site of infection, due to which antibiotics are needed. The use of antimicrobial in the clinical setting was considered a "miracle cure" for dangerous diseases (1). Recent times, overuse of antibiotics have led to the emergence of drug resistance in microbial pathogens. This situation demands identification of novel therapeutic agents which can be used against the drug resistant species. Several bioactive compounds from plant, animal, marine sources have been extensively tested *in vitro* and *in vivo* to elucidate their potential as a antimicrobial agent.

Aggregatibacter actinomycetemcomitans is strongly known to be associated with periodontitis in young adults (2,3) and also with non oral infectious disease such as endocarditis (4). It's prevalence varies widely with geographic location, age, lifestyle and population (5). There are 7 serotypes (a-g) that form genetically divergent lineages (6,7). The mechanisms by which *A. actinomycetemcomitans* cause loss of attachment, are not entirely known. It produces a cytolethal distending toxin which kills host cells like

gingival fibroblasts by blocking their proliferation (8). Vesicles from gram negative bacteria carry out a number of functions including targeted virulence factors and tissues to manipulate host response (9,10). Our team has extensive knowledge and research experience that has translate into high quality publications (11–23),(24–28) (29) (30). The aim of the study is to identify protein targets of *aggregatibacter actinomycetemcomitans* interacting with emodin.

2. MATERIALS AND METHODS:

The study aims to screen protein targets in *A. actinomycetemcomitans* that could possibly interact with emodin. The interaction of those protein targets were analyzed using STITCH V.5 pipeline and the virulence properties of the interacting proteins were deduced by VICMPred and VirulentPred softwares. *A. actinomycetemcomitans* was the organism used and the compound chosen was emodin which was queried using the STITCH database.

The present study follows an observational study design which aims to screen for those proteins or virulence factors interacting with emodin. The STITCH v5.0 pipeline was primarily used for identifying protein interactions; VirulentPred and VICMPred were used for elucidating the virulence property and functional class of the proteins. The subcellular localization of virulent proteins was then assessed using PSORTb v3.0 and the epitopes were identified using BepiPred v1.0 Linear Epitope Prediction.

VICMpred13 and VirulentPred14 pipelines were used for the identification of virulence factors. Virulence factors were screened based on amino acid composition using VirulentPred tool which classified them into two groups, virulent and avirulent. VICMpred groups proteins into four major classes such as, proteins involved in cellular process, metabolism, information storage, and virulence. The overall accuracy of VICMpred and VirulentPred servers were 70.75% and 86%, respectively. The FASTA format of the proteins retrieved from NCBI database were used as an input to run the algorithm.

3. RESULTS AND DISCUSSION:

Epitopes are antigen determining sites for the confirmation of virulent properties. In this study, emodin was the drug that was used to determine its interaction with the *A. actinomycetemcomitans* which is the pathogen. Figure 1 shows the protein interaction network of *A. actinomycetemcomitans* with emodin. Figure 2 shows Epitope prediction (A) 3-deoxy-D-manno-octulosonic acid kinase (B) Aerobic respiration control sensor protein ArcB (C) Hypothetical protein (D) Arabinose 5-phosphate isomerase along with the predicted peptides. The graph depicts the green colour as avirulent and yellow as virulent.

Emodin is a potential compound with antibacterial property against *Aggregatibacter*. Four proteins were identified as virulent in the study, as seen in Table 1. The growth and acid production of *S. mutans* were significantly inhibited by emodin (0.5–2 mg/ml). Emodin also significantly suppressed the synthesis of insoluble glucans by *S. ...* These results suggest that the natural compound emodin may be a novel pharmacological agent for the prevention and treatment of dental caries.

In silico validation is inevitable while choosing a compound to be tested under in vitro and in vivo conditions. It provides clues about the pathway which can be targeted during preliminary screening (31). The present study has been designed to identify the potential interactions with the protein targets with emodin. A study (32) reported the process of inhibition by reserpine where the phytochemical interacts with the transporters of red complex pathogens (33). The proteins were found in the cytoplasm membrane of the bacteria (34).

ABC transporters play a major role in adherence and ATP - binding cassette (35,36). Emodin has antibacterial activity which has been elucidated in several studies (38). Most in vitro studies confirmed anti-

bacterial activity and its mechanisms observed inhibition of DNA replication, cell membrane damage and biofilm formation reduction (39). Although the in silico tools provide preliminary data on the molecular interaction between the compound and protein network of *A. actinomycetemcomitans*, there exists some limitations in the study - experimental approach, Emodin in biological system may not be same and it should target only bacterial protein not host protein so to avoid the undesirable interactions with host proteins, it is imperative to conduct in vitro and in vivo experiments, to gain some clarity on the use of phytochemicals on hosts without any adverse effects (40). Mechanisms observed generally are inhibition of bacterial DNA replication, damage to cell membrane, activity against Plasmids, down regulation of efflux pumps, reduced biofilm formation (41). The mechanisms which leads to the susceptibility of bacteria have to be addressed by performing in vitro experiments to expand the use of such drugs and justify their entry as bactericidal agents. A few limitations of the study are as follows: 1) the drug-protein interactions may be purely physical, that may or may not lead to functional consequences, 2) certain proteins of host may share homology with the bacterial proteins, so the targets should be carefully chosen to avoid adverse effects in the host and 3) the protein interactions evidenced by in silico method may not mimic the type of interactions happening in vivo, as other complex proteins in the vicinity might interfere with the functional pathway confirmed (42).

Further research in this area may also aid in identifying the synergistic and antagonistic effect of these drugs in combination with routine antibiotics, which might open new avenues for handling deadly pathogens in the antibiotic resistant era.

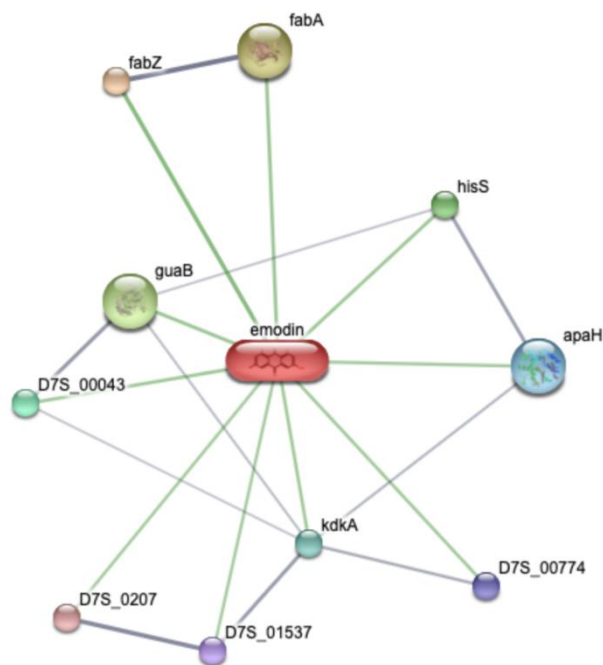
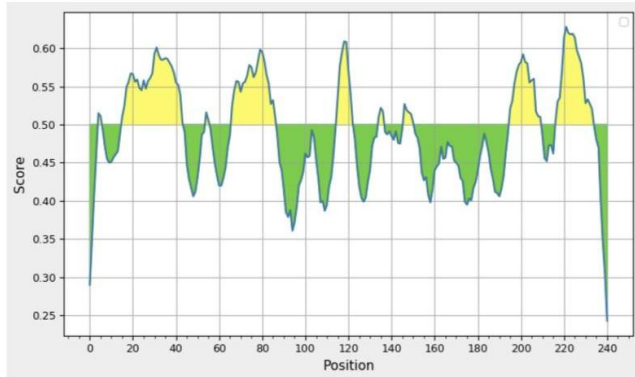


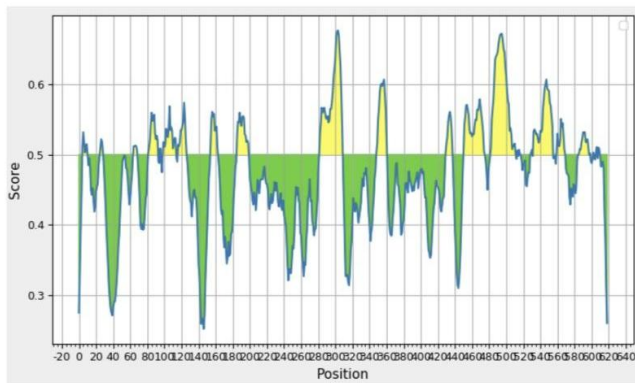
Fig. 1. Protein interaction network of *Aggregatibacter actinomycetemcomitans* with emodin



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	6	QL	2
2	16	44	DQPLANQTQFFFAAFWQQQNRVIGAAGR	29
3	55	56	LF	2
4	67	87	RGGLWGKINKDRYHFSELKNT	21
5	116	123	KGNLGMCY	8
6	135	137	ARD	3
7	147	151	LESTQ	5
8	196	210	CGEKSGRFWKEANLQ	15
9	217	234	NKEAARMHIHFTEQNWQD	18

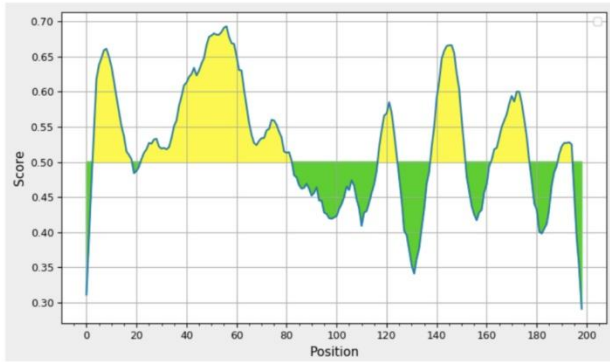
(B)



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	11	KDFVRDF	7
2	26	29	RFSL	4
3	65	69	FGLIS	5
4	82	93	EKLEHSRQALSC	12
5	96	96	E	1
6	99	114	RREVQERVSAEKKLSE	16
7	117	127	DNLEKINRDKT	11
8	155	163	NPSERQONY	9
9	186	199	KIDAKRIELNRKAT	14
10	283	309	IGIVEQDLQKIFELYQAGSDANKSLG	27
11	349	361	AIKPFVEDEHLPL	13
12	430	438	QNYENGVYD	9
13	451	475	VQKKQEYLAQGMDDVIHKPLSLEEL	25
14	483	512	FGEELTQFNLPNPKPQAESVELDTKMLTEL	30
15	514	514	E	1
16	516	517	LG	2
17	530	531	QT	2
18	533	555	QDYVAELQQAAYLNDPHTQPE	23
19	560	569	VHKIKGALAS	10
20	586	599	DTADWQGNIAHWVN	14
21	603	603	K	1
22	606	607	QT	2
23	609	610	VA	2

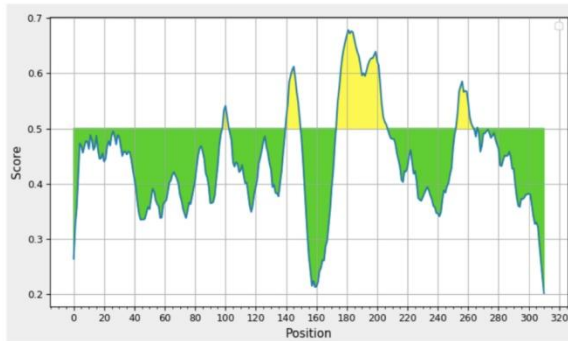
(C)



Predicted peptides:

No.	Start	End	Peptide	Length
1	4	19	SDDKQANLTGLHVLNP	16
2	23	83	LEHQHSSPSSALTDNNRSTHAQSAVNAHSTQSAVNSHRVSSRYEKYDYPGRYQRDEQKQC	61
3	118	125	HINPAFNPQ	8
4	139	152	TGVLEEEAGESGNY	14
5	163	178	HTLWRSPQKPRPIIRG	16
6	190	195	KKSIVI	6

(D)



Predicted peptides:

No.	Start	End	Peptide	Length
1	99	103	ETDDV	5
2	141	150	EREVCPNNLA	10
3	174	207	DFQPEDFAKFPGGSLGRLLCRVKDQMQLHLP	34
4	253	264	NGAETLNKTAQE	12
5	267	267	T	1

Fig. 2. Epitope prediction (A) 3-deoxy-D-manno-octulosonic acid kinase (B) Aerobic respiration control sensor protein ArcB (C) Hypothetical protein (D) Arabinose 5-phosphate isomerase

Table 1: Proteins of *Aggregatibacter actinomycetemcomitans* interacting with emodin.

Organism	Identifier	Proteins which interacts with emodin	VICMPred Functional Class	Virulent Pred	Virulent P r e d Score
<i>Aggregatibacter actinomycetemcomitans</i>	D7S_0123	Beta-hydroxydecanoyl thioester dehydrase	C e l l u l a r Process	Avirulent	-1.024
	D7S_0947	FabA protein	Metabolism	Avirulent	-1.010
	D7S_1931	Histidyl-tRNA synthetase	C e l l u l a r Process	Avirulent	-1.055
	D7S_1159	Diadenosine tetraphosphatase	Metabolism	Avirulent	-0.788
	D7S_0791	Murein transglycosyllase C	Metabolism	Avirulent	-0.339
	D7S_0879	3-deoxy-D-manno-octulosonic-acid kinase	Metabolism	Virulent	0.5992
	D7S_1569	Aerobic respiration control sensor protein ArcB	C e l l u l a r Process	Virulent	1.0580
	D7S_0207	Hypothetical protein	C e l l u l a r Process	Virulent	0.9795
	D7S_0043	Arabinose 5-phosphate isomerase	C e l l u l a r Process	Virulent	1.0306
	D7S_2311	Inosine-5'-monophosphate dehydrogenase	Metabolism	Avirulent	-1.079

4. CONCLUSION:

The study identified the protein targets in *A. actinomycetemcomitans* interacting with emodin through virtual screening, which has to be further validated. This study is the first of its kind which aims in understanding the protein targets against the specific phytocompound.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. *Nat Rev Microbiol*. 2010 Jul;8(7):481–90.
2. Haubek D. The highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans*: evolutionary aspects, epidemiology and etiological role in aggressive periodontitis. *APMIS Suppl*. 2010 Sep;(130):1–53.
3. Henderson B, Ward JM, Ready D. *Aggregatibacter (Actinobacillus) actinomycetemcomitans*: a triple A* periodontopathogen? [Internet]. Vol. 54, *Periodontology 2000*. 2010. p. 78–105. Available from: <http://dx.doi.org/10.1111/j.1600-0757.2009.00331.x>
4. Winkelhoff AJ, Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in nonoral infections [Internet]. Vol. 20, *Periodontology 2000*. 1999. p. 122–35. Available from: <http://dx.doi.org/10.1111/j.1600-0757.1999.tb00160.x>
5. Haubek D, Ennibi O-K, Poulsen K, Væth M, Poulsen S, Kilian M. Risk of aggressive periodontitis in adolescent carriers of the JP2 clone of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* in Morocco: a prospective longitudinal cohort study [Internet]. Vol. 371, *The Lancet*. 2008. p. 237–42. Available from: [http://dx.doi.org/10.1016/s0140-6736\(08\)60135-x](http://dx.doi.org/10.1016/s0140-6736(08)60135-x)
6. Tsuzukibashi O, Saito M, Kobayashi T, Umezawa K, Nagahama F, Hiroi T, et al. A gene cluster for the synthesis of serotype g-specific polysaccharide antigen in *Aggregatibacter actinomycetemcomitans* [Internet]. Vol. 196, *Archives of Microbiology*. 2014. p. 261–5. Available from: <http://dx.doi.org/10.1007/s00203-014-0965-3>
7. Kittichotirat W, Bumgarner RE, Asikainen S, Chen C. Identification of the pangenome and its components in 14 distinct *Aggregatibacter actinomycetemcomitans* strains by comparative genomic analysis. *PLoS One*. 2011 Jul 19;6(7):e22420.
8. Åberg CH, Kwamin F, Claesson R, Dahlén G, Johansson A, Haubek D. Progression of attachment loss is strongly associated with presence of the JP2 genotype of *Aggregatibacter actinomycetemcomitans*: a prospective cohort study of a young adolescent population [Internet]. Vol. 41, *Journal of Clinical Periodontology*. 2014. p. 232–41. Available from: <http://dx.doi.org/10.1111/jcpe.12209>
9. Johansson A. *Aggregatibacter actinomycetemcomitans* Leukotoxin: A Powerful Tool with Capacity to Cause Imbalance in the Host Inflammatory Response [Internet]. Vol. 2, *Toxins*. 2011. p. 242–59. Available from: <http://dx.doi.org/10.3390/toxins2030242>
10. Lally ET, Golub EE, Kieba IR, Taichman NS, Rosenbloom J, Rosenbloom JC, et al. Analysis of the *Actinobacillus actinomycetemcomitans* leukotoxin gene [Internet]. Vol. 264, *Journal of Biological Chemistry*. 1989. p. 15451–6. Available from: [http://dx.doi.org/10.1016/s0021-9258\(19\)84850-0](http://dx.doi.org/10.1016/s0021-9258(19)84850-0)

11. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol.* 2018 Oct;89(10):1241–8.
12. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. *Hypertens Res.* 2020 Jul;43(7):729–30.
13. S G, T G, K V, Faleh A A, Sukumaran A, P N S. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. *Int J Biol Macromol.* 2018 Dec;120(Pt A):876–85.
14. Del Fabbro M, Karanxha L, Panda S, Bucchi C, Nadathur Doraiswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. *Cochrane Database Syst Rev.* 2018 Nov 26;11:CD011423.
15. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. *Hypertens Res.* 2020 Aug;43(8):851–3.
16. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. *Cell Mol Immunol.* 2019 Dec;16(12):935–6.
17. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. *Comput Commun.* 2019 Dec 15;148:176–84.
18. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing Relationship between Patient and Doctor in Public Dental Health using Particle Memetic Multivariable Logistic Regression Analysis Approach (MLRA2). *J Med Syst.* 2018 Aug 29;42(10):183.
19. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. *J Dent Educ.* 2019 Apr;83(4):445–50.
20. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, Characterization and Biological Applications of Biosynthesized Silver Nanoparticles with Chitosan-Fucoidan Coating. *Molecules* [Internet]. 2018 Jun 12;23(6). Available from: <http://dx.doi.org/10.3390/molecules23061429>
21. Alsubait SA, Al Ajlal R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of Different Concentrations of Three Root Canal Sealers on Human Mesenchymal Stem Cells. *Biomolecules* [Internet]. 2018 Aug 1;8(3). Available from: <http://dx.doi.org/10.3390/biom8030068>
22. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from Cuttlefish Bone: Isolation, Characterizations, and Applications. *Biotechnol Bioprocess Eng.* 2018 Aug 1;23(4):383–93.
23. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. *Measurement.* 2019 Mar 1;135:672–7.
24. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. *J Endod.* 2021 Aug;47(8):1198–214.
25. R H, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical

- appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. *Oral Dis* [Internet]. 2021 Jun 21; Available from: <http://dx.doi.org/10.1111/odi.13937>
26. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. *Oral Dis* [Internet]. 2021 Feb 11; Available from: <http://dx.doi.org/10.1111/odi.13798>
 27. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. *Oral Oncol*. 2021 Jun 16;105390.
 28. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. *Oral Oncol*. 2021 Jun 14;105375.
 29. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. *J Ambient Intell Humaniz Comput* [Internet]. 2018 Dec 14; Available from: <https://doi.org/10.1007/s12652-018-1166-8>
 30. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. *Risk Manag Healthc Policy*. 2021 Jul 7;14:2851–61.
 31. Begum S, Naqvi SQZ, Ahmed A, Tauseef S, Siddiqui BS. Antimycobacterial and antioxidant activities of reserpine and its derivatives. *Nat Prod Res*. 2012 Jan 25;26(22):2084–8.
 32. Gao L, Ma Y, Li X, Zhang L, Zhang C, Chen Q, et al. Research on the roles of genes coding ATP-binding cassette transporters in *Porphyromonas gingivalis* pathogenicity [Internet]. Vol. 121, *Journal of Cellular Biochemistry*. 2020. p. 93–102. Available from: <http://dx.doi.org/10.1002/jcb.28887>
 33. Garmory HS, Titball RW. ATP-Binding Cassette Transporters Are Targets for the Development of Antibacterial Vaccines and Therapies [Internet]. Vol. 72, *Infection and Immunity*. 2004. p. 6757–63. Available from: <http://dx.doi.org/10.1128/iai.72.12.6757-6763.2004>
 34. Gibbons S, Udo EE. The effect of reserpine, a modulator of multidrug efflux pumps, on the in vitro activity of tetracycline against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) possessing the tet(K) determinant. *Phytother Res*. 2000 Mar;14(2):139–40.
 35. Li G, Zhang J, Li C, Guo Q, Jiang Y, Wei J, et al. Antimycobacterial activity of five efflux pump inhibitors against *Mycobacterium tuberculosis* clinical isolates [Internet]. Vol. 69, *The Journal of Antibiotics*. 2016. p. 173–5. Available from: <http://dx.doi.org/10.1038/ja.2015.101>
 36. J. SN, Negi J. S, Bisht VK, Bhandari A. K, Bisht D. S, P. S, et al. Quantification of reserpine content and antibacterial activity of *Rauvolfia serpentina* (L.) Benth. ex Kurz [Internet]. Vol. 8, *African Journal of Microbiology Research*. 2014. p. 162–6. Available from: <http://dx.doi.org/10.5897/ajmr2013.5847>
 37. Parai D, Banerjee M, Dey P, Chakraborty A, Islam E, Mukherjee SK. Effect of reserpine on *Pseudomonas aeruginosa* quorum sensing mediated virulence factors and biofilm formation. *Biofouling*. 2018 Mar;34(3):320–34.
 38. Ruiz J, Ribera A, Jurado A, Marco F, Vila J. Evidence for a reserpine-affected mechanism of resistance to tetracycline in *Neisseria gonorrhoeae*. *APMIS*. 2005 Oct;113(10):670–4.
 39. J VP, Vijayashree PJ, SmilineGirija AS, Paramasivam A. *Enterococcus faecalis* an Emerging Microbial Menace in Dentistry-An Insight into the In-Silico Detection of Drug Resistant Genes and Its Protein Diversity [Internet]. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*. 2018.

Available from: <http://dx.doi.org/10.7860/jcdr/2018/36480.12155>

40. Toms L, McQuay HJ, Derry S, Moore RA. Single dose oral paracetamol (acetaminophen) for postoperative pain in adults. *Cochrane Database Syst Rev*. 2008 Oct 8;(4):CD004602.
41. Rani Basu L, Mazumdar K, Kumar Dutta N, Karak P, Dastidar SG. Antibacterial property of the antipsychotic agent prochlorperazine, and its synergism with methdilazine. *Microbiol Res*. 2005 Feb 28;160(1):95–100.
42. Yin Z, Wang Y, Whittell LR, Jergic S, Liu M, Harry E, et al. DNA replication is the target for the antibacterial effects of nonsteroidal anti-inflammatory drugs. *Chem Biol*. 2014 Apr 24;21(4):481–7.

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