

# EFFECT OF ULTRAVIOLET RADIATION ON PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS AND ENTEROCOCCUS

**Running title:** Effect of ultraviolet radiation on pathogenic bacterias.

## ABSTRACT:

**Background:** The study of microorganism is called microbiology, which includes bacteria, viruses, fungi and Protozoa. Microbes play a major role in this field. Microbes are also used for life-saving drugs etc.. The effect of ultraviolet rays on the bacterias are mostly lethal to them, UV is a minor fraction of the solar spectrum reaching the ground surfaces, the UV light radiation will reduce the microbes in the surroundings as well as in the labs.

**Aim:** The aim of this study was to evaluate the effect of the ultraviolet rays on pathogenic bacterias.

**Materials and methods:** Three organisms were selected for the study. *Pseudomonas*, *S. Aureus* and *Enterococcus*. 30 watts Uv tube was used to evaluate the antibacterial activity of the Uv radiation. The exposure time was determined as 5, 10 and 15 mins at a close distance of 10cm. 20 microliter of suspension was taken and mixed in 2 ml of sterile normal saline and exposed for respective duration. Sub culture was done on suitable media after the exposure . Time exposed plates were incubated at 37 degree Celsius overnight and checked for the total CFU and data were tabulated.

**Results:** The Ultraviolet radiation of pathogenic bacteria resulted in a significant total colony forming unit. The result

**Conclusion:** Ultraviolet rays were lethal to the bacterias. There are many airborne bacterias surrounded by environment ultraviolet rays exposure will cause an apparent decrease in the pathogenic bacterias.

**KEY WORD:** Bacteria, Effect, Eco friendly, Pathogenic and Ultraviolet, Innovative technology, Innovative technique.

## **INTRODUCTION:**

The study of microorganisms is microbiology, Which is unicellular, multicellular or acellular. Numerous sub-disciplines of microbiology include virology, bacteriology, protistology, mycology, immunology and parasitology(1,2). Ultraviolet(UV) is a part of the electromagnetic spectrum with a wavelength range 10nm and a related frequency of 400nm, shorter than visible light, but longer than X-rays. In sunlight, UV radiation is present and represents around 10 percent of the overall generation of the electromagnetic radiation from the sun(3). To most humans, ultraviolet rays are invisible. The human eye's lens blocks most radiation in the 300-400nm wavelength range; the cornea blocks shorter wavelengths(4). Ultra-violet lamps are used in biological and surgical equipment to sterilize work spaces and instruments, Ultraviolet radiation is used to destroy undesirable microorganisms in some food systems. UV can be used for pasteurizing fruit juice using an ultraviolet medium of high strength and disinfection using UV radiation is commonly used in wastewater treatment applications(5).

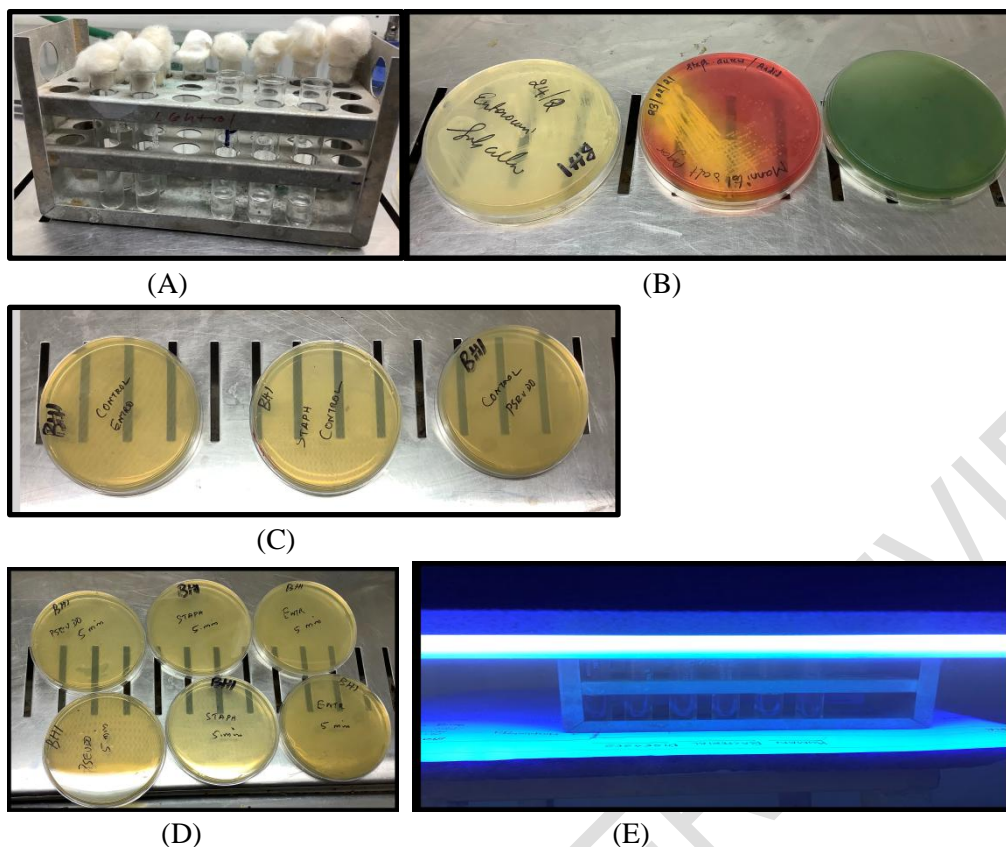
The classical use of the ultraviolet has occurred in biological safety in laboratories, in recent years it is also used for the inactivation of the growth of microorganisms(6). By forming dimers in RNA and DNA, ultraviolet light inactivates microorganisms that interfere with the transcription and replication(6). The adoption of UV lamps, such as ozone generating low pressure Hg vapour lamps, can be an effective means of disinfecting the microorganisms in the air. As a light source, many current infection prevention devices use low pressure mercury vapour lamps. This is a low-cost source of high-energy photons. Pulsed xenon light source technology has recently emitted a large spectrum of light(7). Some important Pathogenic bacteria are salmonella species, listeria monocytogenes, Escherichia coli and staphylococcus aureus etc., are espoused in the uv rays(8). Escherichia coli is an indigenous bowel of healthy people and warm-blooded animals and accounts for about

1% of the global biomass of bacteria(9). *Pseudomonas aeruginosa* is a common, Gram-negative, rod-like shape bacteria that also causes disease in humans, plants and animals(10). Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have been extensively used in home and hygiene settings in disinfectants and biocidal materials. While the principal bactericidal effect of biocides has been reported for cytoplasmic membrane integrity disruptions, little is known about the biochemical changes caused by such chemical agents(11).

Photocatalytic sterilisation by UV light illumination with a wavelength of 254 nm is one of the standard methods of sterilisation, which provides a high rate of sterilisation at room temperature(12). The mechanism of microorganisms' UVC inactivation is to trigger cell damage by causing changes in the chemical structure of DNA chains(13). The effect is the development of cyclobutane pyrimidine dimers (CPDs) that cause DNA molecule distortion, which may cause cell replication malfunctions and lead to cell death(14). Ultraviolet radiation prevents the infection and diseases transmitted by the pathogenic bacteria through air(15). The aim of this study is to evaluate the effect of ultraviolet radiation on pathogenic bacterias.

#### **MATERIALS AND METHODS:**

Three organisms were selected for the study. *Pseudomonas*, *S. Aureus* and *Enterococcus*. 30 watts Uv tube was used to evaluate the antibacterial activity of the Uv radiation. The exposure time was determined as 5, 10 and 15 mins at a close distance of 10cm. 20 microliter of suspension was taken and mixed in 2 ml of sterile normal saline and exposed for respective duration. Sub culture was done on suitable media media after the exposure . The subcultured plates were incubated at 37 degree Celsius overnight and checked for the total CFU and data were tabulated.



**Figure 1:** This figure represents [A] denotes bacterial suspension, [B] denotes bacterial organisms, [C] denotes control culture plates, [D] denotes culture plates of bacteria and [E] denotes UV exposure on pathogenic bacterias.

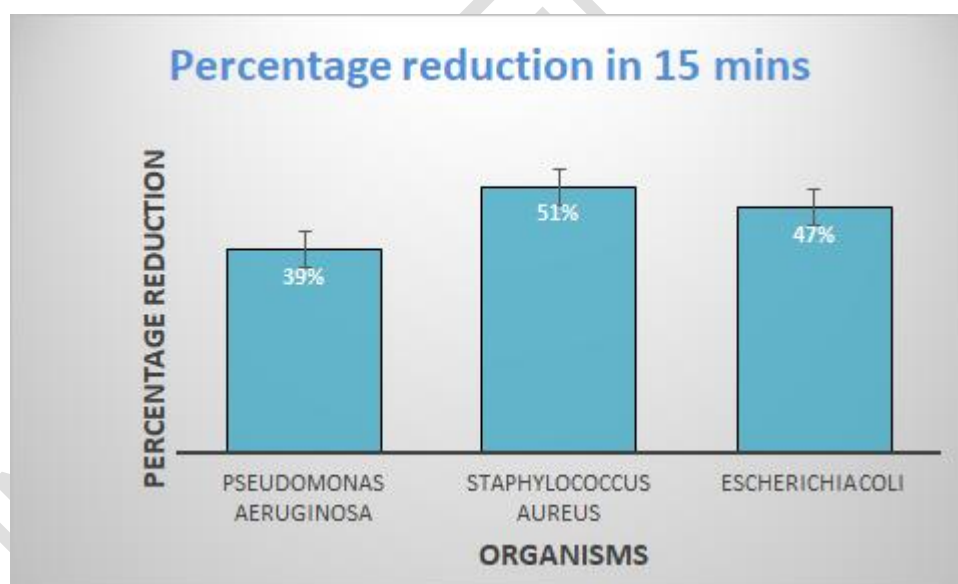
## RESULTS:

**Table 1:** This table represents the total colony forming units of susceptible organisms tested.

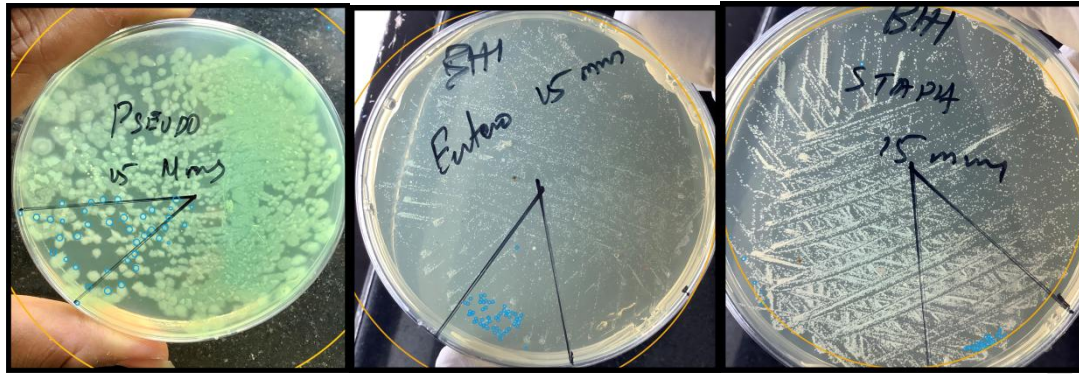
Timings	5 minutes	10 minutes	15 minutes	Control
<b>Pseudomonas Aeruginosa</b>	<b>1448</b>	<b>1000</b>	<b>550</b>	<b>1400</b>
<b>Staphylococcus aureus</b>	<b>800</b>	<b>700</b>	<b>656</b>	<b>1280</b>
<b>Escherichia coli</b>	<b>720</b>	<b>680</b>	<b>684</b>	<b>1456</b>

**Table 2:** This table represents the percentage reduction of bacteria in 15 minutes.

ORGANISMS	PERCENTAGE REDUCTION IN 15 MINUTES
<i>Pseudomonas Aeruginosa</i>	39%
<i>Staphylococcus aureus</i>	51%
<i>Escherichia coli</i>	47%



**Figure 2:** This figure represents the percentage reduction of *Pseudomonas aeruginosa*, *S. aureus* and *E. coli* in 15 minutes. X axis refers to bacterial organisms and Y axis refers to the percentage reduction in 15 minutes.



**Figure 3:** This figure represents the pathogenic bacterial colonies which were formed after the exposure of UV radiation for 15 minutes.

## DISCUSSION:

The main goal of this study is to prove that Ultraviolet radiation treatment for disinfecting the pathogenic bacteria in hospitals and laboratories. It did not show much difference in the reduction of total colony forming units. Table 1 summarizes the total colony forming unit of all bacteria i.e., *S. aureus*, *Pseudomonas aeruginosa* and *E. coli*. Table 2 summarizes the percentage reduction of bacteria in 15 minutes. A detailed description about the percentage reduction of bacteria in 15 minutes is described as a bar chart in Figure 1. The results showed that the decreasing order of pathogenic bacteria gets disinfected with the ultraviolet radiation treatment in the order of *Pseudomonas aeruginosa* > *Escherichia coli* > *Staphylococcus aureus*. The population of foodborne pathogens on chicken decreased by an increased UV-C radiation dose had been proved by the study done by Chun et al., that supports the present study(16). Previous study done by Pereire et al., proved that With the ultimate mean IgG decline by about 50 percent, the UVC treatment of colostrum dramatically lowered Ig G concentration(6). The study done by Thai et al., concluded that ultraviolet C radiation was lethal to the organism like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* supports present study(17). Previous study proved that This knowledge also indicates that UV light at 254 nm is a bactericidal substance for *S. aureus* and *E. faecalis* antibiotic resistant strains even as brief as five seconds and enterococcus bacteria are more likely to destroy UV. The findings, indicating that the inactivation of a large number of medically significant bacteria (including the *Staphylococcus aureus* methicillin-resistant) indicate the potential for a new decontamination system for a variety of applications with a narrow, 405-Nm spectrum visible lighting from an LED source(18).

Research has shown that UVCs can cause DNA damage somewhat to mammalian cells at successful antimicrobial doses. The damage to UVC-induced DNA has also been found,

however, to be repaired quickly by DNA enzymes. There has also been research into the role of UVC irradiation in wound healing and variable outcomes(19). There has also been research into the role of UVC irradiation in wound healing and variable outcomes. While pathogens prevent the healing of infected wounds, UVC microorganisms may be expected to improve the healing of wounds in infected wounds(20). Previous study by Walker et al., concluded that UV rays can serve to prevent transmission of respiratory viral diseases through an effective control measure(21). The high UV sensitivity of aerosol coronavirus suggests that UV disinfection is an effective tool for preventing significant respiratory viral diseases such as SARS(21). For the increase in radiation dose at a given wavelength, *e. coli* cells reduce exponentially. Furthermore, the radiation dose required to kill a particular portion of the *E. coli* cells increases exponentially with increased radiation wavelength(22) As UV disinfection is more common in drinking water and in clinical and industrial facilities, the bactericidal effects of UV and visible light on this bacterial species should be investigated(23). Our team has extensive knowledge and research experience that has translate into high quality publications(24–35),(36–40)

The limitation of this study is that it is confined to a limited number of culture plates and organisms. The study was conducted using 3 organisms in about 18 culture plates for each time interval and 3 culture plates for control. The future scope of this study is that it can be done on large scale culture plates by using various pathogens that are present in hospitals and laboratories.

## **CONCLUSION:**

The germicidal treatment of UV rays was used on three different organisms (*Pseudomonas*, *E.coli*, *staphylococcus* ).UV light at safe wavelength to be used is to reduce the colony count as it is not too strong and has a high efficiency in eradicating microorganisms. But higher radiation of UV light is lethal to pathogenic bacteria, this was proved by this present study. Among the 3 bacterial organisms, *Pseudomonas aeruginosa* showed a quick percentage in reduction in about 15 minutes. Therefore, UV light can be a method to control microorganisms and there is no complete reduction of numbers although microorganisms maybe can repair this damage. The efficacy of the ultraviolet radiation will be proportional to bacterial load in the system and the effect is based on the availability on the surface.

## **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. McGenity T, van der Meer JR, de Lorenzo V. Handbook of Hydrocarbon and Lipid Microbiology. Springer; 2010. 4699 p.
2. Timmis K, Cavicchioli R, Garcia JL, Nogales B, Chavarría M, Stein L, et al. The urgent need for microbiology literacy in society. *Environ Microbiol.* 2019 May;21(5):1513–28.
3. Nakano S, Miyata A, Kizawa J, Kurosaka D, Miyata K, Oshika T. Blue light–filtering and violet light–filtering hydrophobic acrylic foldable intraocular lenses: Intraindividual comparison [Internet]. Vol. 45, *Journal of Cataract and Refractive Surgery.* 2019. p. 1393–7. Available from: <http://dx.doi.org/10.1016/j.jcrs.2019.05.027>
4. Booth MG, Jeffrey WH, Miller RV. RecA Expression in Response to Solar UVR in the Marine Bacterium *Vibrio natriegens* [Internet]. Vol. 42, *Microbial Ecology.* 2001. p. 531–9. Available from: <http://dx.doi.org/10.1007/s00248-001-1009-5>
5. Amin MM, Hashemi H, Bina B, Movahhedian Attar H, Farrokhzadeh H, Ghasemian M. Pilot-scale studies of combined clarification, filtration, and ultraviolet radiation systems for disinfection of secondary municipal wastewater effluent [Internet]. Vol. 260, *Desalination.* 2010. p. 70–8. Available from: <http://dx.doi.org/10.1016/j.desal.2010.04.065>
6. Pereira RV, Bicalho ML, Machado VS, Lima S, Teixeira AG, Warnick LD, et al. Evaluation of the effects of ultraviolet light on bacterial contaminants inoculated into whole milk and colostrum, and on colostrum immunoglobulin G. *J Dairy Sci.* 2014 May;97(5):2866–75.
7. Szeto W, Yam WC, Huang H, Leung DY. The efficacy of vacuum-ultraviolet light disinfection of some common environmental pathogens. *BMC Infect Dis.* 2020 Feb 11;20(1):127.
8. Kim D-K, Kim S-J, Kang D-H. Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts [Internet]. Vol. 97, *Food Research International.* 2017. p. 280–7. Available from: <http://dx.doi.org/10.1016/j.foodres.2017.04.009>

9. Leclerc H, Mossel DAA, Edberg SC, Struijk CB. Advances in the Bacteriology of the Coliform Group: Their Suitability as Markers of Microbial Water Safety [Internet]. Vol. 55, Annual Review of Microbiology. 2001. p. 201–34. Available from: <http://dx.doi.org/10.1146/annurev.micro.55.1.201>
10. Dai T, Tegos GP, St Denis TG, Anderson D, Sinofsky E, Hamblin MR. Ultraviolet-C irradiation for prevention of central venous catheter-related infections: an in vitro study. *Photochem Photobiol.* 2011 Jan;87(1):250–5.
11. Aboulizadeh E, Bumah VV, Masson-Meyers DS, Eells JT, Hirschmugl CJ, Enwemeka CS. Understanding the antimicrobial activity of selected disinfectants against methicillin-resistant *Staphylococcus aureus* (MRSA). *PLoS One.* 2017 Oct 16;12(10):e0186375.
12. Nerandzic MM, Cadnum JL, Pultz MJ, Donskey CJ. Evaluation of an automated ultraviolet radiation device for decontamination of *Clostridium difficile* and other healthcare-associated pathogens in hospital rooms. *BMC Infect Dis.* 2010 Jul 8;10:197.
13. Penno K, Jandarov RA, Sopirala MM. Effect of automated ultraviolet C–emitting device on decontamination of hospital rooms with and without real-time observation of terminal room disinfection [Internet]. Vol. 45, American Journal of Infection Control. 2017. p. 1208–13. Available from: <http://dx.doi.org/10.1016/j.ajic.2017.06.015>
14. Dai T, Vrahas MS, Murray CK, Hamblin MR. Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? [Internet]. Vol. 10, Expert Review of Anti-infective Therapy. 2012. p. 185–95. Available from: <http://dx.doi.org/10.1586/eri.11.166>
15. Mahmoudi H, Bahador A, Pourhajibagher M, Alikhani MY. Antimicrobial Photodynamic Therapy: An Effective Alternative Approach to Control Bacterial Infections [Internet]. Vol. 9, Journal of Lasers in Medical Sciences. 2018. p. 154–60. Available from: <http://dx.doi.org/10.15171/jlms.2018.29>
16. Chun HH, Kim JY, Lee BD, Yu DJ, Song KB. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage [Internet]. Vol. 21, Food Control. 2010. p. 276–80. Available from: <http://dx.doi.org/10.1016/j.foodcont.2009.06.006>
17. Anuchin AM, Mulyukin AL, Suzina NE, Duda VI, El-Registan GI, Kaprelyants AS. Dormant forms of *Mycobacterium smegmatis* with distinct morphology. *Microbiology.* 2009 Apr;155(Pt 4):1071–9.
18. Conner-Kerr TA, Sullivan PK, Gaillard J, Franklin ME, Jones RM. The effects of ultraviolet radiation on antibiotic-resistant bacteria in vitro. *Ostomy Wound Manage.* 1998 Oct;44(10):50–6.
19. Bijl M, Kallenberg CGM. Ultraviolet light and cutaneous lupus [Internet]. Vol. 15, Lupus. 2006. p. 724–7. Available from: <http://dx.doi.org/10.1177/0961203306071705>
20. Bak J, Ladefoged SD, Tvede M, Begovic T, Gregersen A. Dose requirements for UVC

- disinfection of catheter biofilms [Internet]. Vol. 25, Biofouling. 2009. p. 289–96. Available from: <http://dx.doi.org/10.1080/08927010802716623>
21. Walker CM, Ko G. Effect of ultraviolet germicidal irradiation on viral aerosols. *Environ Sci Technol*. 2007 Aug 1;41(15):5460–5.
  22. Vermeulen N, Keeler WJ, Nandakumar K, Leung KT. The bactericidal effect of ultraviolet and visible light on *Escherichia coli* [Internet]. Vol. 99, Biotechnology and Bioengineering. 2008. p. 550–6. Available from: <http://dx.doi.org/10.1002/bit.21611>
  23. Gray NF. Biology of Wastewater Treatment [Internet]. Series on Environmental Science and Management. 2004. Available from: <http://dx.doi.org/10.1142/p266>
  24. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen *A. baumannii* and related species [Internet]. Vol. 94, Archives of Oral Biology. 2018. p. 93–8. Available from: <http://dx.doi.org/10.1016/j.archoralbio.2018.07.001>
  25. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol*. 2019 Dec;90(12):1441–8.
  26. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. *Hypertens Res*. 2020 Feb;43(2):153–4.
  27. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. *Heliyon*. 2018 Dec;4(12):e01051.
  28. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. *Cell Mol Immunol*. 2020 Jun;17(6):668–9.
  29. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. *Cell Mol Immunol*. 2020 May;17(5):550–1.
  30. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? *Front Immunol*. 2020 May 27;11:1206.
  31. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. *Hypertens Res*. 2020 Jan;43(1):74–5.
  32. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. *Nat Prod Res*. 2021 Jun;35(11):1893–8.
  33. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from

*Porphyromonas gingivalis* with the bioactive compounds from *Rosmarinus officinalis*. *Asian Biomed* . 2019 Oct 1;13(5):197–203.

34. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences* [Internet]. 2020;82(2). Available from: <https://www.ijpsonline.com/articles/targeting-nm23h1mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html>
35. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An in-silico approach. *Acta Virol*. 2020;64(1):93–9.
36. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent*. 2021 May;31(3):436–41.
37. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent*. 2021 Mar;31(2):285–6.
38. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol*. 2021 Jun;126:105132.
39. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? *J Dent Sci*. 2020 Dec;15(4):562–3.
40. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. *Oral Health Prev Dent*. 2020 Apr 1;18(1):379–86.