



Industrial experience - An opinion article

Can Natural Structural Analogues of Antimicrobials be Repurposed as External Use Disinfectants? A Small Scale Industry's Experience in India During Pandemics

Juluri R. Rao

Environmental Microbiology, Agri-Food & Biosciences Institute
Newforge Lane, Belfast BT9 5PX, UK

ABSTRACT

This opinion article highlights the experience of a small group of textile entrepreneurs in India, who despite the economic downturn during the pandemic crisis, and diversified to a small scale healthcare industry. By not only having innovated a way out of their set-backs, but also utilising a proof of concept industry-led study, they have repurposed macromolecular natural product structural analogues for external use applications such as protective antimicrobial coating for domestic and healthcare fabric laundering, and as disinfectant in places with a heavy footfall (e.g. mass / rapid transport systems, hospitality sector) in developing nations. Furthermore, natural antimicrobial analogues supplied at nanomolar dosage, this prototype product adds no burden to either environmental toxicity or antimicrobial resistance. A collaborative interplay of the industry with academia and medical researchers would heighten the science of this industries unique approach of a different kind of 'drug repurposing' to underpin a practical working solution for long-term disinfection beyond the contagion pandemics to both public and healthcare utilities in economically challenged developing nations.

Key words: Repurposed oral drugs; Structural analogues of natural product Antimicrobials; External use non-alcoholic disinfectants formulations; Industrial experience in a developing nation

INTRODUCTION

Over the last 12 years, coming from an environmental microbiologists looking glass, I have been involved in a raft of projects with my interactive colleagues comprising clinical microbiologists, healthcare practitioners at Northern Ireland Public Health laboratories, School of Experimental Medicine, Queens University, Belfast and biomolecular, biomedical scientists at University of Ulster featuring in my citations, and last but not the least valuable input by scientists from international universities (e.g. Japan, China). We were essentially focussed on exploring both the knowledge on herbal medicines [1] to fight antimicrobial resistance (AMR) [e.g. 2, 3], and seek potential alternative complementary medicines for combating the AMR menace that is costing our healthcare within UK. This aspect has unequivocally been at the heart of our scouting for the local native plant and microbial resources which provided novel insights into plant health and biomedical applications [4]. The natural product antimicrobial constituents were assessed using standard protocols as well as novel bioimaging techniques [5] for antagonistic microbe-microbe interplay and/or antibiosis [e.g. 6-9], antimicrobial efficacies

against clinical pathogens [10-14], and antibiotic effects of native plant and fungal protein/peptide extracts [e.g. 15]. More recently we demonstrated that structural analogues of plant phytohormone (gibberellins) showed inhibitory effects at nanomolar concentrations of their enantiomers upon a wide range of clinical and plant pathogens (16).

INDUSTRIAL EXPERIMENTING AND EXPERIENCES – TRIALS AND TRIBULATIONS

“Crisis is a time for innovation”

To this end, in this opinion article I wish to bring a small scale industry's efforts who were former textile industry specialists in South of India and were experiencing serious setbacks in their own fabric trading due to / during the height of pandemic lockdowns and factory closures. The small scale industry followed the mantra “recession is a time for innovation” [17] and as to why it is more important for small-medium businesses particularly to focus on diversifying into healthcare innovation and as a launch-pad out of the covid-19 crisis [18]. Thus, undaunted by such challenges as the covid-driven nuances to their fabric businesses, the small scale industry launched a new start up small scale healthcare industry, India (SSHI) www.lokkavach.com with a view to ‘re-invent’ their textile expertise and twinning it with the idea of formulating an environmentally sustainable, health and safety compliant new disinfectant formulations that may be targeted for use as antimicrobial coating for daily use fabrics as well as disinfectant conditioner whilst bulk laundering in healthcare attires, and as surface disinfectant in public transport environments. To this end, the small scale industrialists in India, prompted in part by my recent evidence based research in natural product structurally active antimicrobials sought my scientific advice, and in the light of their synchronous feasibility studies of ‘repurposing’ naturally occurring structural analogue chemical entities for external disinfection purposes, purportedly at health and safety compliant and environmentally safe nanomolar levels.

Proof of concept of a small scale start up industry in healthcare

In a proof of concept, industry-led study, the SSHI produced a new formulation YUDEF (under non-disclosure, trade secret and patent pending agreements). From the industry's generic disclosures available to me at the time of writing this opinion article, it contains ca. 250-400 ppm of Quaternary Ammonium Compounds aka QACs for short. However, it is a well-documented fact that QACs are generic disinfectant agents are cheap, and extensively used against environmental contagions [e.g. 19], but lesser effective against spore formers and viruses. Thus the innovative step by the industry-led study involved fortifying the QACs with naturally occurring (organic) compounds referred to as AVC^R (Anti-Viral Composites of natural product structural analogues) for exhibiting robust contagion defence against stubborn bacteria, fungi and also expressing antiviral effects. These were sourced via structural biology methods [e.g. 20] for matching the chemical analogues of approved drug with proven broad spectrum antimicrobial efficacies. As a small scale non-medical industry, they regarded this step to be a practicable fast-track approach than to be trialling via high throughput, cost-intensive screening route on 1000s of potential candidates. For a prototype product, the choice was narrowed to a handful of macromolecules not limited to but inclusive of peptides, aromatic amines, lactones etc. naturally occurring macromolecular ‘structural repurposed drug analogues’.

***In vitro* study upon the 'anti-viral' performance of 'repurposed drug analogues'**

An *in vitro* study upon the 'anti-viral' performance of 'repurposed drug analogues' incorporated in face masks' at nanomolar (ca 10^{-5} mM) concentration and fabric pieces supplied by SSHI, was conducted at a local vaccines and viruses research centre, Tamil Nadu Veterinary and Animal Sciences, Chennai, India (TANUVAS) using a type species of corona virus (Rat Coronavirus) offering such testing of disinfection products services at the time serial lockdowns in India due to covid-19 pandemics in 2020. With apparent restrictions for testing covid-19 itself as a strain at the time, the proof of concept of the efficacy of the repurposed YUDEF product study was conducted on L2p.196 cell line, which is susceptible for rat coronavirus infection. A preliminary cytotoxicity study was also performed on the SSHI's formulation. Concentrations lower than the one causing cytotoxicity was taken up for *in vitro* study. The chosen concentration was mixed with $10^{4.3}$ TCID₅₀ of virus in equal volumes and infected on to cell cultures. The concentration that caused complete abrogation of virus-induced cytopathic effects i.e. CPE (implying a reduction of 4.0 log of virus infectivity or 99.9% disinfecting efficiency) was considered as minimal disinfection concentration. The cytotoxicity of SSHI's formulation was also ascertained using a spray of the virus on an impervious surface followed by anti-viral formulation treatment. The dried up surface was washed and assessed for the presence of infectious virus. The antimicrobial tests on YUDEF product were compliant to EU accreditation tests set at EN14476 and ISO 18184 guidelines for virus reduction (up to >99%) on coated soft fabric and hard surfaces, and ascertained to be non-toxic (Biotech report, supplementary data). The Irish Equine Centre www.irishequinecentre.ie, one of the few in Europe offering antiviral testing services for such prototype products on fabrics during the pandemics; they corroborated independently that the YUDEF product has strong antiviral efficacy. Together with other laboratory test reports (supplementary data pdf) provide unequivocal evidence that the SSHI's natural prototype product drug analogue repurposed product (YUDEF) possesses broad spectrum antimicrobial protection on fabrics when challenged via a range of standard microbial inhibitory assays and testing procedures.

DISCUSSION**Drug repurposing model for oral use**

From the very outset, the SSHI have taken a cue from their 'big brother' business leaders such as pharma companies. For instance, in order to contend with this global health crisis situation, an almost twin track strategy operates via the development of several effective anti-SARS-CoV-2 vaccines which are in many ways a re-invention in its molecular medicine value [e.g. mRNA vaccines (Pfizer, AstraZeneca, Moderna among others)], and repurposed drugs ["The Repurposing, Focused Rescue, and Accelerated Medchem" (ReFRAME)] approach. This binary policy will no doubt contribute to the control of SARS-CoV-2 strains global origin variants (e.g. alpha, beta, gamma, delta, the latest omicron etc.). On-going intense efforts to identify therapeutic interventions and their efficacies, fitness for purpose initially identified simple antibiotics e.g. Hydroxychloroquine, Azithromycin [21] for *in vitro* covid-19 neutralisation effects. A plethora of higher precision chemical entities (ca. 12,000 small-molecules [22] also augur well for real-time covid patient treatment. Other repurposing of FDA approved drugs include monoclonal antibody (MABs) (e.g. Casirivimab/Imdevimab as intravenous (IV) or subcutaneous (SC) drugs (Roche), Molnupiravir, (Merck, Sharp and Dohme (MSD) and Ridgeback Biotherapeutics, Paxlovid (Pfizer), oral tablet form of drugs repurposed for covid treatment.

A potential gap in innovative repurposing approach for external use disinfectants

Persistence of coronaviruses on soft and hard surfaces

At the start of the pandemics in 2020, scientists around the world were in their initial estimates as to how long the novel coronavirus (covid-19) remains active on the surface. It is well known that hard and soft material surfaces such as fabrics, doorknobs, countertops, and electronic equipment can transmit viral and bacterial diseases. For instance the information available for SSHI indicated in one study [24] that similar coronaviruses could persist on hard surfaces like glass, metal or plastic up to 9 days while another [25] found SARS-CoV2 variants could remain on plastic and stainless steel for up to 3 days. No doubt with the benefit of the hindsight, a plethora of cited articles on this topic are afloat now, but one may try to envision the 2020 pandemic bleak horizon scanning and limited information by the industry (SSHI) in India.

Virus shell and its susceptibility and/or resistance to external disinfectants

Many gastrointestinal viruses like norovirus which have a tough protein shell called a capsid, viruses with this fatty wrapping are relatively vulnerable. The essence of this and other scientific findings when simplified to the small scale industrialists is that COVID-19 is much more sensitive, and that enveloped viruses like SARS-CoV-2, which rely on a protective lipid coating are the easiest type to deactivate by alcohol-based products that can rapidly disintegrate the protective lipids. This knowledge was largely used for managing surface contaminations by alcoholic (70%) as antiviral agents for external use, followed by a number of harsher chemical alternatives. Non-alcoholic agents was led by quaternary ammonium disinfectants (QACs), commonly used in health-care and food-service industries, that attack protein and lipid structures, thwarting the pathogen's typical mode of infection but have lower efficacies on robust spore formers and stubborn viruses. Harsher products bleach, other potent oxidizers swiftly break down a virus's essential components too but leave more eco-toxic residues and their costly implications to the environment. From the SSHI's information search on their new products during the first phase of pandemic outbreak in 2020, they came across an EPA disinfection information update [23]: On March 3, 2020, the EPA, USA and EU later, released a list of antimicrobials that can be used for surface control of covid-19, under an emerging viral pathogens program and that the *makers of disinfectants* can request approval to claim a product is expected to kill a particular virus based on its ability to kill similar viruses as 'external use only'. Taking this as a broad guideline, the SSHI in India have thus understandably tested their prototype product (YUDEF) against Rat coronavirus and Equine Arteritis Virus (EAV) as a means of demonstrating the virus kill efficacy (supplementary pdf data).

A spot light on hygiene versus disinfection in developing nations

The disinfection strategic focus is particularly highly relevant in developing and poorer countries where the general hygiene and infection prevention in their daily life is a formidable challenge. Often sanitation standards are sadly inadequate in general hospitals with a heavy foot fall comprising the ailing public and the untiring primary healthcare providing carers alike. Often the most frequently encountered situations include soft surfaces such as people's attires, fabrics at home (possibly due to their less frequent automated washing and repeated re-use habits), as well as in their healthcare setting (e.g. healthcare worker attire, wound dressings, apparels, bed linens, bed-bed privacy curtains), and semi-hard surfaces (e.g. upholstered furniture, door knobs, glass, plastic), and in the busy in-patient and visitor footfall prone healthcare hospitals than in the developed world. Despite the advantages of using alcohol based formulations in a developing nations, it poses logistical problems of large volumes of alcohol

based disinfectants for external use. Also, there is a discernible scarcity of alcohol-free “viral disinfection agents for external use” strategies in primary healthcare (hospitals, clinics) and common public environments (e.g. mass / rapid transport systems, hospitality sector) in developing nations.

Re-inventing and/or re-purposing of a different kind of disinfectant

To this end, the SSHI in India were obviously focussing on one hand, as to how they can learn from the ‘repurposing of drugs for oral use’, and vis-à-vis focus on key pointers from EPA’s disinfectant program guidelines to set out their starting points, milestones and goals to achieve their objective of producing a health and safety compliant non-alcoholic disinfectant, yet different from those known to date. The net sum of all plusses and minuses led them to explore the utilization of a non-alcoholic QACs as a base component that gives a generic sanitation of a broad spectrum of environmental bacterial, fungal and viral contagions, and fortify the QACs with a natural product ingredient that provided unique antiviral effects. Given the above backdrop, the SSHI have not only innovated a way out of their set-backs, but they have also re-invented (or effectively repurposed) an affordable formulation for soft and hard surfaces using a nanomolar structural analogues having matching molecular contours and conformation offering antimicrobial efficacies against microbial contagions including providing the crucial viral control efficacies. Unsurprisingly their face mask coated product was outcompeted in retail markets saturated with top corporate brands selling antiviral nano-coated (e.g. Ag) or nano-filter fabric masks. The available information provided by the SSHI indicated YUDEF theoretically poses negligible environmental burden of unwarranted antimicrobial resistance; it has desirable molecular ion anchoring towards QACs, target innate cations of fabric / hard surface matrices. However, the exact mechanism of YUDEF’s inhibition of bacteria, fungus or virus is yet to be elucidated. This QAC based formulation (YUDEF) fortified with nanomolar antivirals, is in their view offered a simple, cheap, locally sourced, environmentally sustainable means of external disinfection utility than harsher chemical disinfectant options to combat the on-going waves of COVID-19 pandemics in many economically challenged South East Asian countries.

The industry of course, still have to go through a maze of other chemical registration nuances, and formidable challenges await them as their current formulation has to meet national and international product regulatory standards and licensing protocols / requirements.

CONCLUSION

The challenges and opportunities of a small scale local fabric industry may be commendably viewed as ‘thinking globally’ on public health and ‘acting locally’ to combat pandemic contagions. This industry’s experience is an example of developing new range of antiviral disinfectant parallel to the oral antiviral repurposing discoveries by big drug companies. Of course this is not a solve-all disinfectant. A collaborative interplay of the industry with academia and pharmaceutical researchers will therefore help service industries (disinfectants, textile) to underpin the science behind deriving a practical working solution to both public and healthcare utilities in the continuing challenges the contagions pose world over. Such prototype indigenous products may help economically challenged highly populated nations to comply with some reasonable hygienic forms of home management, and to concomitantly maintain personal and community hygiene practices including in their primary healthcare scenarios in the era of post pandemic emergence cycles of global viral variants.

ACKNOWLEDGEMENTS

My sincere thanks to all my interactive colleagues and co-authors cited in publications 1-16, 19 comprising clinical microbiologists, healthcare practitioners at Northern Ireland Public Health laboratories, School of Experimental Medicine, Queens University, Belfast and biomolecular, biomedical scientists at University of Ulster featuring in my citations, and last but not the least valuable input by scientists from international universities (e.g. Japan, China) is gratefully appreciated. The supplementary data cited in this article is available with www.lokkavach.com.

References

- [1] Millar B. C. Moore J.E., and Rao, J. R. *Fighting antimicrobial resistance (AMR): Chinese herbal medicine as a source of novel antimicrobials – an update*, *Letters in Applied Microbiology* 2021. 73(4) p 400-407. doi: 10.1111/lam.13534 <https://doi.org/10.1111/lam.13534>
- [2] Nelson, D., J. E. Moore and J. R Rao. *Antimicrobial resistance (AMR): Significance to food quality & safety*, *Food Quality and Safety*, 2019. 3(1) p 15–22. <https://doi.org/10.1093/fqsafe/fyz003>
- [3] Malnarcic, C.M., et al., *Quality of antibiotics in destinations*, *Travel Medicine and Infectious Disease*, 2020. 38 p 1-3. <https://doi.org/10.1016/j.tmaid.2020.101747>
- [4] Nelson, D. *Antimicrobials: Novel insights into plant health and biomedical applications*. PhD Thesis. University of Ulster, Northern Ireland, U.K. 2017.
- [5] Moore, J. E., et al., *Description of a simple bio-imaging technique to assess inhibition/growth promoting properties of novel agents on moulds*. *British Journal of Biomedical Science* 2010. 67(3) p 145-146. <https://doi.org/10.1080/09674845.2010.11730312>
- [6] McIlroy, R., et al., *Discovery of inhibition of Burkholderia cenocepacia, Pseudomonas aeruginosa and Stenotrophomonas maltophilia by the Brown Rot Basidiomycete Fungus, Postia placenta*, *Ulster Medical Journal*, 2021. 90(3) p 168-174. https://www.ums.ac.uk/090_3.html
- [7] McIlroy, R., et al., *Pseudomonas aeruginosa – Candida interplay: effect on in vitro antibiotic susceptibility of Pseudomonas aeruginosa when grown in the presence of Candida culture*, *British Journal of Biomedical Science*, 2020. 77(4) p 1-5. <https://doi.org/10.1080/09674845.2020.1819003>
- [8] Millar, B.C., et al., *Antimicrobial properties of Basidiomycota macrofungi to Mycobacterium abscessus isolated from patients with Cystic Fibrosis*, *International Journal of Mycobacteriology*, 2019. 8(1) p 93-97. https://pubmed.ncbi.nlm.nih.gov/30860186/#:~:text=PMID%3A%2030860186,10.4103/ijmy.ijmy_167_18
- [9] Nelson D., et al., *Antimicrobial properties of native Ulster macrofungi (mushrooms and toadstools) to clinical pathogens*. *Ulster Medical Journal* 2019. 88 (2) p 128-132. ANTIMICROBIAL PROPERTIES OF NATIVE ULSTER MACROFUNGI (MUSHROOMS AND TOADSTOOLS) TO CLINICAL PATHOGENS (nih.gov)
- [10] Rao, J. R., et al., *Antimicrobial properties of shiitake mushrooms (Lentinula edodes)*. *International Journal of Antimicrobial Agents* 2009. 33(6) p 591-592 <https://doi.org/10.1016/j.ijantimicag.2008.10.018>
- [11] Hearst, R., et al., *An examination of the antibacterial and antifungal properties of constituents of shiitake (Lentinula edodes) and oyster (Pleurotus ostreatus) mushrooms*. *Complementary Therapies in Clinical Practice* 2009. 15(1) p 5-7. <https://doi.org/10.1016/j.ctcp.2008.10.002>
- [12] Woods-Panzaru, S., *An examination of antibacterial and antifungal properties of constituents described in traditional Ulster cures and remedies*", *Ulster Medical Journal*, 2009. 78(1) p 13-15. <http://www.ncbi.nlm.nih.gov/pmc/articles/pmc2629014/>
- [13] Moore, P. J., et al., *Examination of the antibacterial properties of sphagnum moss (Sphagnum spp.) and its significance with turf burning in Ireland*, *British Journal of Biomedical Science* 2012. 69(4) p 178-18 [https://pubmed.ncbi.nlm.nih.gov/23304795/#:~:text=4\)%3A178%2D80.,PMID%3A%2023304795,-Copy](https://pubmed.ncbi.nlm.nih.gov/23304795/#:~:text=4)%3A178%2D80.,PMID%3A%2023304795,-Copy)
- [14] Efstratiou, E., et al., *Antimicrobial activity of Callendula officinalis petal extracts against fungi, as well as Gram-ve and Gram+ve clinical pathogens*. *Complementary Therapies in Clinical Practice* 2012. 18(3) p 173-176. <https://pubmed.ncbi.nlm.nih.gov/22789794/#:~:text=PMID%3A%2022789794,j.ctcp.2012.02.003> [15] Hearst, M., et al., *An investigation into antimicrobial properties of protein extractions from native fungal and plant species*,

Journal of Pharmacognosy and Phytotherapy 2010. 2(8) p 103-107.

<http://www.academicjournals.org/jpp/abstracts/abstracts/abstracts2010/Dec/Hearst%20et%20al.htm>

[16] Toner, P., et al., *Antimicrobial properties of phytohormone (gibberellins) against phytopathogens and clinical pathogens*. Access Microbiology, 2021. 3 (10). DOI 10.1099/acmi.0.000278
<https://doi.org/10.1099/acmi.0.000278>

[17] Chesborough, H., Recession is a time for innovation, 2010. Science Business Newsletter Recession is a time for innovation | Science|Business (sciencebusiness.net)

[18] Fursenthal, L., et al., 2020. Healthcare innovation: Launchpad out of the covid-19 crisis Innovation in a crisis: Why it is more critical than ever | McKinsey

[19] Moore, J.E. and Rao, J.R. Efficacy of biocide Steri-7 against the common Gram negative bacterial pathogens *Burkholderia cenocepacia*, *Burkholderia gladioli*, *Burkholderia multivoran* and *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* associated with cystic Fibrosis (CF). British J Biomed Science 2013. 70(2) p 80-81.
<https://doi.org/10.1080/09674845.2013.11669940>

[20] Renaud, J-P *Structural Biology in Drug Discovery: Methods, Techniques, and Practices*, ed2020, pp Minneapolis, MN, United States, (John Wiley & Sons, Inc.). 600 DOI:10.1002/9781118681121.

[21] Liu, J. *et al.*, *Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro*, Nature Communications, 2020. *Cell Discov* 6, 16. <https://doi.org/10.1038/s41421-020-0156-0>
www.nature.com/celldisc

[22] Bakowski, M.A. et al., *Drug repurposing screens identify chemical entities for the development of COVID-19 interventions*. Nature Communications, 2021. <https://doi.org/10.1038/s41467-021-23328-0>

[23] Anonymous, *Disinfectant use and Covid-19: list of antimicrobial products for use against SARS-CoV-2*, 2020, Disinfectant Use and Coronavirus (COVID-19) | US EPA

[24] Kampf, G., et al., *Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents*, 2020. Journal of Hospital Infection 104 (3) 246–251. DOI: 10.1016/j.jhin.2020.01.022

[25] van Doremalen et al., *Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-2*, *New England Journal of Medicine* 382:1564-1567 DOI: 10.1056/NEJMc2004973