Protocatechuic Acid; a Green Chemical Natural Disinfectant.

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Title: Protocatechuic Acid; a Green Chemical Natural Disinfectant.

Eligibility criteria is met for the **Small business Award.**

Focus Area 3: Design of greener chemicals. Protocatechuic acid (PCA) is a green chemical reagent, presently categorized as biocide by EPA. PCA is native to the ecosystem, everywhere in nature; soil, water ways, all plants, fruit and vegetables. PCA is common to the human diet. The bacteria in the human bowel manufacture a small amount daily.

Most recent Milestones are the critical discoveries resulting in US patents as a broad spectrum antibacterial, a biofilm destroyer for MRSA and Pseudomonas and recently the discovery of COVID19 anti-viral properties of PCA. The work is original and novel as evidenced by two of the multiple US patents related to PCA.

10,004,705	7/26/2018. *This patent included the metabolites of protocatechuic acid and 2,4,6 trihydroxybenzaldehyde for the **destruction of biofilms of MRSA and Pseudomonas** on metal, cloth and ceramic.*
10,959,969	3/30/2021. **The treatment of COVID19 with protocatechuic acid**. *This patent is for treating the patient; oral, intravenous, injection, etc. We cannot say this without some clinical study.*

Publication: *Jalali, Omid; Best, Molly; Wong, Alison; Schaeffer, Brett; Bauer, Brendon; Johnson, Lanny. Protocatechuic Acid as a Topical Antimicrobial for Surgical Skin Antisepsis. Preclinical Investigations. JBJS Open Access: July-September 2020 - Volume 5 - Issue 3 - p e19.00079 doi: 10.2106/JBJS.OA.19.00079.*

Jalali, Omid; Best, Molly; Wong, Alison; Schaeffer, Brett; Bauer, Brendon; Johnson, Lanny. Reduced Bacterial Burden of the Skin Surrounding the Shoulder Joint Following Topical Protocatechuic Acid Application Results of a Pilot Study. JBJS Open Access d 2020:e19.00078. http://dx.doi.org/10.2106/JBJS.OA.19.00078

The US Component: The inventor is an US citizen. All the research was performed at independent contract laboratories in USA. The assembly of the commercial product is in all independent contact USA GMP facilities.

Abstract:

PCA is a candidate for the Green Chemistry Challenge as a disinfectant for hard surface fomites in any and all facilities; i.e. medical, transportation, hospitality and military. PCA is a small molecule, a biocide by EPA designation.

PCA in this clinical application is intended to replace short acting disinfectants and those having any potentially toxicity; i.e. triclosan or quaternary ammoniums. PCA is safe by every measure. PCA is natural and found throughout the environment; soil, plants, trees, rivers and lakes, fruits and vegetables. It is common to the human diet. It has been designated at Generally Recognized As Safe (G.R.A.S.) by FDA as a food flavoring. PCA's human pharmacokinetics and pharmacodynamic are known. PCA is non-toxic, non-allergenic and non-mutagenic. PCA is non-inflammable and has no fumes.

The implementation is eminent, awaiting EPA approval. The raw material is available in 1000-kilogram quantities. Quality control verification is made at independent laboratories. There are

multiple manufacturing facilities in USA. Distribution channels are in place. There is a known ready market waiting purchase.

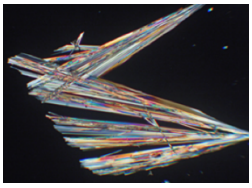
The quantitative benefit to the environment of this alternative natural reagent is not easily calculated. However, the PCA substitution would eliminate the use of any and all disinfectants with toxins; i.e. triclosan or quaternary ammoniums.

In practice, PCA is easily soluble in a water (up to 1.24 %) or in an alcohol (up to 25%) for delivery. As low as 0.25% will invisibly coat glass surfaces. A 1% solution is most common for most applications. Higher concentrations may be used on ventilation filters for removing aerosol bound virus. The delivery method of PCA may be varied; i.e. spray or wipe.

Upon delivery to a hard surface, the vehicle evaporates leaving an invisible, uniform, confluent, enduring miniscule anti-microbial barrier coating of PCA crystals physically adhered to the fomite surface.

For illustration purposes, excess concentrated PCA coating was purposely applied to a filing cabinet. At two years, the crystals were scraped with the fingernail. Note the 2x4 mm area, not to scale and pile of crystals to the right.

The dry PCA crystal has a unique physical structure. Unlike salt, zinc and some antibiotics that are rhomboid and smooth surfaced, PCA crystal has sharp spear like projections.



10X photomicrograph of PCA sharp crystals under polarized light.

Mode of Action: The microbe body is physically disrupted upon contact with PCA's sharp crystalline protrusions coating on the fomite. This duration is known effective at 48 hours with MRSA and Pseudomonas biofilms and at 24 hours for SARSCoV2 virus.

PCA if ever discharged, the amounts would be small. No necessity for additional water and being native to the environment, will not add pollution.

PCA's potential toxicity to the waste water is unlikely due to minimal discharge from the fomite. Das, et al concern for low amounts of PCA being toxic is refuted by studies showing same amounts to be anti-bacterial and causing accelerated healing of mammalian wounds. The use of PCA will not produce any carbon dioxide nor require any extra use of energy.

Scope of the program

The use of protocatechuic acid (PCA) crystalline coating of hard surfaces in facilities is a safe and effective method to disinfect bacterial and viral pathogens.

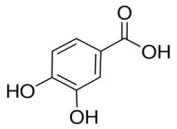
Broad Spectrum Antibiotic: In vitro evidence that PCA is a broad-spectrum antibiotic was determined by the Kirby-Bauer disc diffusion method. US patent 9,498,413.

Biofilm Destroyer: The biofilm destroying properties of PCA for MRSA and Pseudomonas was a 99.999% kill over a 48 period of continuous flow of live biofilms over metal and cloth coated with PCA crystals via the by ASTM E-2647 Drip Flow Biofilm Reactor method. US patent 10,004,705.

Anti-Viral: The anti-viral supporting evidence was by in vitro laboratory testing that replicates the intended clinical use method. The articles of metal, plastic and material from N95 mask were spray coated with PCA in liquid vehicle. Upon evaporation, the residual dry PCA crystalline coating was subject to SARS CoV2 virus droplet contact. The results were 90%+ inactivation of the virus at 10 and 60 minutes as well as 24 hours after application. US patent 10, 959, 969.

Dose Dispersion: The dose of PCA is small, dispersed widely over the fomites in a facility. The PCA so delivered adheres to the fomite with minimal discharge unless intentionally washed off. Even if used in multiple facilities in a given geographical location results in miniscule amounts discharged compared to that amount already throughout the environment. This application thereby eliminates introduction of hazardous substances and hazards to public health that may exist with toxic reagents presently in use; triclosan or quaternary ammoniums.

Chemical Nature: PCA is a small molecule, a benzoic acid 2,4 di-hydroxybenzaldehyde. The CAS number is 99 50 3.



PCA exists throughout nature and the addition of miniscule amounts by the proposed mitigation methods will not be a pollutant, but only add small amount to the existing PCA ecological presence.

PCA crystalline properties exist in dry or an aqueous vehicle thereby retaining the primary anti-microbial properties.

Realized Benefits and Drawbacks

PCA is a known powerful anti-oxidant which is common to health and wellness. 10X more powerful than vitamin E.

PCA is known anti-inflammatory. Inflammation is common denominator of all disease.

Benefits: As show in the patents above, PCA is an anti-bacterial, biofilm destroying and anti-viral for encoated virus; i.e. SARS-CoV-2 when applied to an article of hard or cloth surface.

Safety: PCA has been recognized as safe for ingestion by FDA as a food flavoring additive. PCA has an existing FDA G.R.A.S. designation as Generally Recognized As Safe. http://www.ift.org/~/media/Food%20Technology/pdf/2009/06/0609feat_GRAS24text.pdf

PCA is Common to the Human Diet: You can and do eat it. In fact, you consume it every day in your diet. PCA is ingested daily in various amounts and foods. The following is a list and amounts of PCA in common foods.
http://phenol-explorer.eu/contents/polyphenol/412

The bacteria in the human bowl produce a small amount daily.

Human Health and Environmental Benefits: The proposed clinical application is one of disinfection of potential pathogens by spray, misting or wipe application to fomites; hard surfaces, rooms, facilities and clinical environments. After spraying a surface with alcohol and or a water vehicle containing small amounts of PCA and following evaporation an invisible residue of confluent PCA crystals on the surfaces as illustrated above.

Concerns: The potential inflammability is nil with the dry crystalline residue. The same with water. There is a temporary potential inflammability prior to rapid evaporation when alcohol is the vehicle.

Das, et al mentioned PCA in waste water is a potential concern. PCA's potential toxicity to the waste water was supported by in vitro studies by Babich, et al. Babich expressed concern for as little 5-25mM concentrations of PCA being toxic to human cells. This contention is countered by in vivo evidence of PCA's anti-bacterial properties and accelerated healing of mammalian wounds with 0.25 mM in a water vehicle. This evidence and the low discharge amount should remove this concern. The use of PCA will not produce any carbon dioxide nor require any extra use of energy.

Furthermore, PCA rapidly biodegrades, in as short a time as 24 hours. This is known in human metabolism and in tissue culture. Its fate in waste water is likely the same.

Pilot Plant Exists: The reagent PCA is available from many international sources. There are many US GMP plants available for manufacture into a commercial product. The following are used at the present time; Samson Pharma, City of Commerce, CA and QYK Brands, Garden Grove, CA and CoreChem of Knoxville, TN.

Manufacture: PCA is presently biochemically manufactured, thereby eliminating the trace metals found in previous production by extraction from plant material. Certificate of Analysis accompanies each production which is subsequently verified by independent laboratory for purity.

EPA application initiated in Jan 2020. Still under review.

Technology Comparisons: PCA is not found in other disinfectants. PCA is native to the environment. It is safe by any measure. PCA is non-toxic, non-allergenic and non-mutagenic. Although prices are rising due to shipping costs plus world-wide inflation the cost of goods for PCA is relatively low, such to be commercially viable and competitive with the existing market.

Performance has been established in vitro which in this case exactly replicates the clinical application; a PCA spray on hard surfaces of metal, plastic, and fabric. Positive results were for bacteria and SARS CoV2 virus, which incidentally is an encoated virus which is inactivated by PC physical disruption. The later had same results at two separate independent contract laboratories; MRIGlobal and Illinois Institute of Technology.

The broad application is assured by societal and humanitarian unmet need, the product availability and the small amounts of PCA released over time into the environment following disinfectant practices.

Planned Commercialization: Commercialization awaits an EPA application that was submitted 23 months prior to this writing. The reagent is available in 1000-kilogram quantities. The manufacturing facilities are in place.

Technology was launched under emergency use as sanitizer spray.

The Commercial Products: PCA is combined with distilled or sterile water in concentrations of 0.25-1.24% for most common applications. Note that 1.24% is maximum solubility in water. PCA may be combined with either 70% isopropyl alcohol or 60% ethanol in concentrations of 1% (varies from 0.25 up to 25% for selected specific applications).

Science and Innovation: The application is original, and determined novel as evidence by the multiple US patents. This is further evidenced by the US patent 10, 969, 959 was issued 7-month after submission of application, because no prior art. Publications at peer reviewed Journal of Bone and Joint Surgery exists for basic science and skin disinfectant. (see above). The mode of action is known.

Human Metabolism: The pharmacokinetics and pharmacodynamics are known; much already in medical literature.

Mode of action: The anti-bacteria and anti-viral modes of action are well known in the literature and by evidence supporting US patents.

Bacterial Mode of Action: Inhibitory mechanisms of PCA on bacteria growth are wide-ranging and include

- destabilizing the bacteria cytoplasmic membrane,
- altering the permeability of the bacteria plasma membrane,
- inhibiting extracellular microbial enzymes,
- directly altering microbial metabolism, and
- depriving microbes of substrates required for growth [1].

Specifically, PCA can;

- change bacterial physicochemical surface properties. For example, ferulic acid has been shown to decrease hydrophobicity of Pseudomonas aeruginosa [2]. PA treatment can also
- alter bacterial polarity by changing bacteria surface electron acceptors on both gram-positive (increased acceptor components) and gram-negative (decreased acceptor components) strains [2].
- It has been shown that as PA concentrations increase, the percentage of cell membrane damage significantly increases, as indicated by release of intracellular K+, with greater effects observed with gram-negative bacteria strains than with gram-positive bacteria strains [2]

1: Dietrich, H.; Pour Nikfardjam, M.S. Influence of Phenolic Compounds and Tannins on Wine-Related Microorganisms. In Biology of Microorganisms on Grapes, in Must and in Wine; König, H., Unden, G., Fröhlich, J., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 421–454, ISBN 978-3-319-60021-5.

2. Borges, A.; Ferreira, C.; Saavedra, M.J.; Simões, M. Antibacterial Activity and Mode of Action of Ferulic and Gallic Acids Against Pathogenic Bacteria. Microb. Drug Resist. 2013, 19, 256–265.

Anti-Bacterial Duration of Action: The duration of action of PCA crystalline coated metal and polyester was a minimum of 48 hours with an unprecedented 99.999% destruction MRSA and Pseudomonas.

PCA'S Anti-Viral Mode of Action is the **Host Targeting Principle**: The PCA crystal by its inherent sharp physical structure (see above) physically disrupts the surface of the bacteria or an encapsulated virus upon contact. PCA therapeutic effect is initiated by physical disruption of the virus coating. This is in contrast to other non-physical biological methods for capsular disruption.

Importance of the physical disruption of the viral coating in Host Targeting: Jackman and Cho described the lipid coating as the "common denominator" of all enveloped viruses; a group that includes flaviviruses, alphaviruses, coronaviruses, filoviruses, retroviruses and more. No other shared feature exists broadly across all those diverse viruses, which is why he proposes host-targeted antivirals might have greater potential as pandemic-preparedness tools.

Jackman JA, Shi P-Y, Cho N-J. Targeting the Achilles Heel of Mosquito-Borne Viruses for Antiviral Therapy. ACS Infectious Diseases 2019 5 (1), 4-8DOI: 10.1021/acsinfecdis.8b00286

Following the physical disruption, the other antimicrobial properties of PCA become active.

The crystal structure of the reagent causes physical disruption of bacterial and viral coating resulting in bacteria death and viral inactivation.

Biological Disruption: The medical literature substantiates the following known antiviral properties are found in PCA. These take effect in addition to the physical disruption.

- low pH; p*K*a of 4.49.

Dawson, R. M. C.; et al. (1959). Data for Biochemical Research. Oxford: Clarendon Press.

- anti-protease
- anti-docking reagent or docking inhibitor.

Elsbaey, et al report included that PCA bound well to multiple targets for SARS CoV-2 virus, including Mpro and PLpro.

Elsbaey M, Ibrahim MAA, Bar FA, Elgazar AA. Chemical constituents from coconut waste and their in silico evaluation as potential antiviral agents against SARS-CoV-2. S Afr J Bot. 2021 Sep;141:278-289. doi: 10.1016/j.sajb.2021.05.018. Epub 2021 May 28. PMID: 34092840; PMCID: PMC8162769.

Inhibitor of Mpro.

> Acar reported "PCA to have a binding affinity of -4.9 kcal/mol and effective against Mpro. The docked pose of protocatechuic acid with main protease (7BQY) and ligand interaction of protocatechuic acid with 7BQY." PCA had a Log*P* of 0.88 which means it was easily absorbed. With a positive CYP34, PCA would have capacity to cross the blood brain barrier.

Acar A. Evaluation of phenolic acids of Corylus avellane L. as a potential SARS CoV-2 Main protease inhibitors. Erzincan University. Journal of Science and Technology. 2021, 14 (2), 492-509. DOI: 10.18185/erzifbed.897348.

- cellular and hormonal immunity.

Guo, et al concluded that PCA effect was due to enhanced cellular and hormonal immunity.

Guo Y, Zhang Q, Zuo Z, et al. Protocatechuic acid (PCA) induced a better antiviral effect by immune enhancement in SPF chickens. Microb Pathog. 2018; 114:233-238. doi: 10.1016/j.micpath.2017.11.068.

Anti-Viral Duration of Action: The independent contract laboratory in vitro testing at MRIGlobal and Illinois Institute of Technology showed a PCA crystalline coating on an article of metal, plastic and material from N95 mask inactivated the SARS CoV-2 virus upon contact at 10 and 60 minute and at minimum of 24 hours following dry crystalline coating on the article.