Inflammation-mediated Deglucuronidation and Promiscuous-binding Behavior of Polyphenols for Antiviral Assaying and Trialing with Relevance to COVID-19

Abstract

The Covid-19 pandemic has elicited much laboratory and clinical research attention on vaccines, mAbs, and certain small-molecule antivirals against SARS-CoV-2 infection. By contrast, there has been comparatively little attention on plant-derived compounds, especially those that are understood to be safely ingested at common doses and are often commonly consumed in the diet. With broader scope for assays and trials against a diverse array of viral infections, we review elucidations of the pharmacokinetic mechanisms of polyphenolic compounds over the past two decades and survey their putative frequent-hitter behavior. We find that many polyphenols are indeed promiscuous binders, suggesting a candidate mechanism of non-specific inhibition combined with inflammation-targeting specificity. As such a mechanism poses a possible pathway of inhibiting viral replication uniquely in infected tissue, we highlight pre-clinical studies of polyphenols that indeed reduce virion replication. It is hoped that greater awareness of the potential specificity of polyphenolic activation to sites of pathogenic infection will spur renewed research and clinical attention on assaying and trialing against several infectious viral diseases.

1. Introduction

Currently approved therapies for infection by SARS-CoV-2, the etiological agent of the COVID-19 pandemic, include monoclonal antibodies and certain repurposed drugs such as dexamethasone. Monoclonal antibody therapy suffers from difficult logistics to administer, and the approved repurposed drugs have only modest effect on disease outcome. Some novel therapeutics developed for coronaviruses[1,2] as well as further repurposed drugs[3] are under regulatory review as antivirals for SARS-CoV-2. Each has its own strengths but also drawbacks such as perceived concerns about mutagenicity, time to production ramp-up, or side-effect profile.

Persistently low worldwide vaccination rates, the potential for breakthrough infections, and the ability for vaccinated individuals to achieve viral loads sufficient to infect others[4], suggest that there remains ample scope for a replication-inhibiting antiviral in the panoply of pandemic-alleviating healthcare tools.

Natural products may present a potentially untapped source of antiviral activity. Plants must resist viruses whose constituent peptides are restricted to the same twenty proteogenic amino acids. Plant virus proteins share similar fundamental constraints on protein secondary and tertiary structure as viruses with mammalian hosts. Plants' secondary metabolites are known particularly for plant-protection. Prevalent among the secondary metabolites are polyphenols. One of the three primary polphyenol classes are flavonoids[5].

Flavonoids are a family of over eight thousand unique compounds that provide several advantages to plants.[6–8] These compounds are responsible for some pigment and aroma of flowers and fruits, thereby attracting pollinators.[9–11] Various flavonoids also protect plants from both biotic and abiotic stressors,[9,12,13] providing antimicrobial defenses,[9,11,14] acting as UV filters,[9,11,15] and serving as signaling molecules.[9,11,16] Further, despite sparse literature on the topic, several flavonoids are also demonstrated to inhibit several plant viruses.[17–21]

Recent research has demonstrated antiviral modes of activity for flavonoids by targeting neuraminidase[22,23], proteases[23–25] and DNA/RNA polymerases.[24]

A frequent target of coronavirus antivirals is the SARS-CoV-2 main protease, owing especially to the successful history of protease inhibitors on reducing HIV replication. Several polyphenols showed potent antiviral activity to SARS-CoV's main protease.[26–30] Among these, the polyphenolic flavonoid hesperetin was unique in potently inhibiting the action of the main protease in cell-based assay.[26] Hesperetin dose-dependently inhibited cleavage activity of the 3CLpro in expressed in Vero E6 cells with an IC50 of 8.3 μM.[26]

However, polyphenols like hesperetin are disfavored by industrial medicinal chemists for proceeding through the hit-to-lead (H2L) stage of the drug discovery pipeline.[31,32] Polyphenols are categorized among the Pan-Assay INterference compounds (PAINs)[33] (other terms are "frequent-hitters", "promiscuous inhibitors", "privileged structures/scaffolds", and "invalid metabolic panaceas"[34]), and are suggested to obscure the results of various assays. They also bind broadly to assays' protein targets themselves.

Due to the ongoing pressing need for further COVID treatment strategies, we review the pharmacokinetic and putative frequent-hitting behavior of polyphenols' as a class with an eye toward ascertaining 1) their potential as an antiviral 2) whether or not polyphenols simultaneously should pose risks to ordinary healthy cellular processes.

2. What defines a polyphenol?

While IUPAC has defined the term "phenols"[35], a definition of polyphenols remains yet to be formally accepted. Quideau (2011) explored definitions of polyphenols extensively, providing an applicable description:

> "*The term "polyphenol" should be used to define plant secondary metabolites derived exclusively from the shikimate-derived phenylpropanoid and/or the polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression*."[5]

In describing polyphenols in part based on their provenance provides excellent exclusivity. However, one could question whether it is helpful to exclude phenols such as acacetin which have only one phenolic ring. For large-scale cheminformatic purposes, which challenge the application of biosynthesis pathway criteria, an applicable definition may be to treat polyphenols as any molecule with more than one phenolic ring but lacking elements other than C, H, and O.

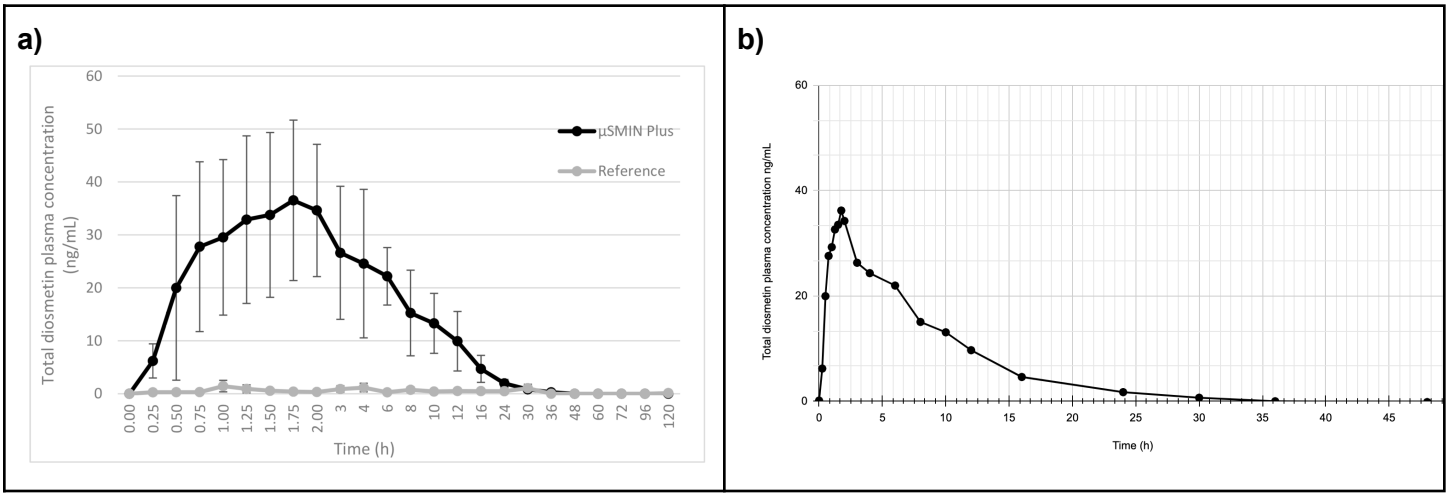
3. Poor polyphenol PK perception

The therapeutic efficacy of any antiviral whose purpose is to reduce viral replication requires maintaining a efficacious concentration of the ligand at its putative target for an extended period of time. Conservatively, this period should ideally be of long duration relative to a virus's replication time to reach peak viral load. An interval typically measured in days in the case of SARS-CoV-2 infection in humans[36].

However, polyphenolic compounds' potential for efficacy for any particular pathology is criticized due to a prima facie poor pharmacokinetic ADMET profile. Consider, for example, diosmetin. A primary intermediate metabolite of the pharmaceutical formulation known as Daflon (comprised of 90% diosmin, and 10% hesperidin, diosmetin, linarin, and isorhoifolin), it and similarly proportioned formulations are prescribed in many countries around the world for chronic venous insufficiency (CVI).

Ingested diosmin becomes diosmetin through Phase I metabolism through the intestinal wall, and then is either glucuronidated or sulfated through Phase II metabolism in the liver.[37–47] Serum analysis on healthy individuals demonstrates negligible presence of the aglycone in plasma, and low sustained levels of the diosmetin conjugates (primarily glucuronides) in plasma, with t_{max} of 2.3 hrs and $t_{1/2}$ ranging from 8-70 hrs. [38–42,44–47] Stachulski and Meng (2013) and Tranoy et al. (2014) note that most glucuronides are rapidly eliminated by the kidneys, posing an apparent limitation to their efficacy.[48,49] Glucuronidation further reduces bioavailability to the intracellular compartment as the glucuronide moiety imparts a hydrophilicity that prevents cellular uptake.[49] Russo et al. (2018)[39] provide a prototypical example of flavonoid plasma pharmacokinetics, which are reproduced and linearized in Figure 1a and b, respectively.

Figure 1 - diosmetin plasma concentration (ng/mL) vs time:



a) Diosmetin plasma concentration (as ascertained following deglucuronidation), after administration of a 50mg/kg micronized diosmin formulation to rats. Image licensed under CC by 4.0 from Russo et al. (2018)[39]
b) same plot with linearized axes.

Yet to our knowledge 1) no quantitative bioavailability assays of diosmetin have taken place in non-plasma compartments such as extracellular fluid and tissue in humans; 2) tissue distribution studies of flavonoids in animal models are few. More in vivo distribution data to support therapeutic insights into polyphenols would be valuable.

4. Drawbacks of polyphenol PK analysis

On broader review of the polyphenol pharmacokinetic literature, five insights about pharmacological assays emerge:

1. The most commonly obtained pharmacological assay for concentration of polyphenols or their metabolites is blood plasma analysis, rather than interstitial fluid or intracellular fluid.[37–47,50–59]
2. A polyphenol's plasma concentration profile alone provides no data on tissue distribution or biotransformation[50,59,60]
3. It is very difficult to sample intracellular fluid for drug/metabolite concentration profiling to the exclusion of extracellular and serum fluid.[61,62]
4. Even radiolabeled assaying of all possible elimination routes fails to provide a complete accounting of polyphenol dosage intake.[59]
5. Plasma samples of polyphenols are more frequently obtained from healthy individuals, rather than those suffering from a particular pathology.[37–47,51–59]

Therefore if any particular pharmaceutical candidate's PK profile achieves significant distribution in organs other than those associated with either the GI tract or renal tract, it would be unascertainable from serum analysis alone. Further, if any particular pathology has an effect on a compound's tissue distribution (whether by causing sequestering in sanctuary sites, or adduct formation with the target in tissue both of which represent an increase in the volume of distribution), then plasma analysis alone remains poorly positioned to provide the relevant readout. Rather, tissue analysis in sacrificed animal models, or comprehensive radiolabeled elimination quantitation in humans, would be required.

Walle et al. (2001) demonstrated such a radiolabeled analysis[59]. Notably, they found that carbon dioxide was a major metabolite of quercetin in humans,[59] suggesting a rarer elimination pathway than typically encountered by pharmacological analysis. Even with this exotic elimination route taken into account, the full dose of quercetin was not always accounted for. One can speculate that sequestration of quercetin products in tissue compartments was maintained past the 72-hr study period.

Moreover, while DeBoer et al. (2005)[63] and Bieger et al. (2008)[64] demonstrated that quercetin reaches certain tissues other than those associated with GI and renal tracts in healthy animal models, even these studies still fall short of addressing any putative bioavailability of flavonoids uniquely to tissue affected by diseased circumstances.

5. The flavonoid paradox

To begin addressing these pharmacokinetic challenges, we look at a subtle but critically important aspect of the pharmacokinetic profile of polyphenols such as flavonoids as ascertained from the literature.

Menendez et al. (2012) and Perez-Vicaino et al. (2013) posed the problem of the "flavonoid paradox".[65,66] The paradox is summarized by the observation that several flavonoid polyphenols have been shown to demonstrate therapeutic effects for various pathologies in vivo, and yet their pharmacological profiles suggest poor bioavailability with rapid plasma clearance.

The paradox is resolved by the following deconjugation mechanism: During inflammation (as happens during infection of several etiologies), phagocytes arrive at the extracellular fluid surrounding the sites of inflammation. The phagocytes express β-glucuronidase which accomplishes deglucuronidation (also known as deconjugation) of the flavonoid glucuronide into its aglycone form. The deconjugated flavonoid aglycone subsequently diffuses through the cell membrane where they can reach their target. The mechanism is summarized in Table 1. For purposes of this review only, the mechanism steps are labeled stages B, C, D, E, and F for clarity.

Table 1 - Deglucuronidation-through-inflammation mechanism steps

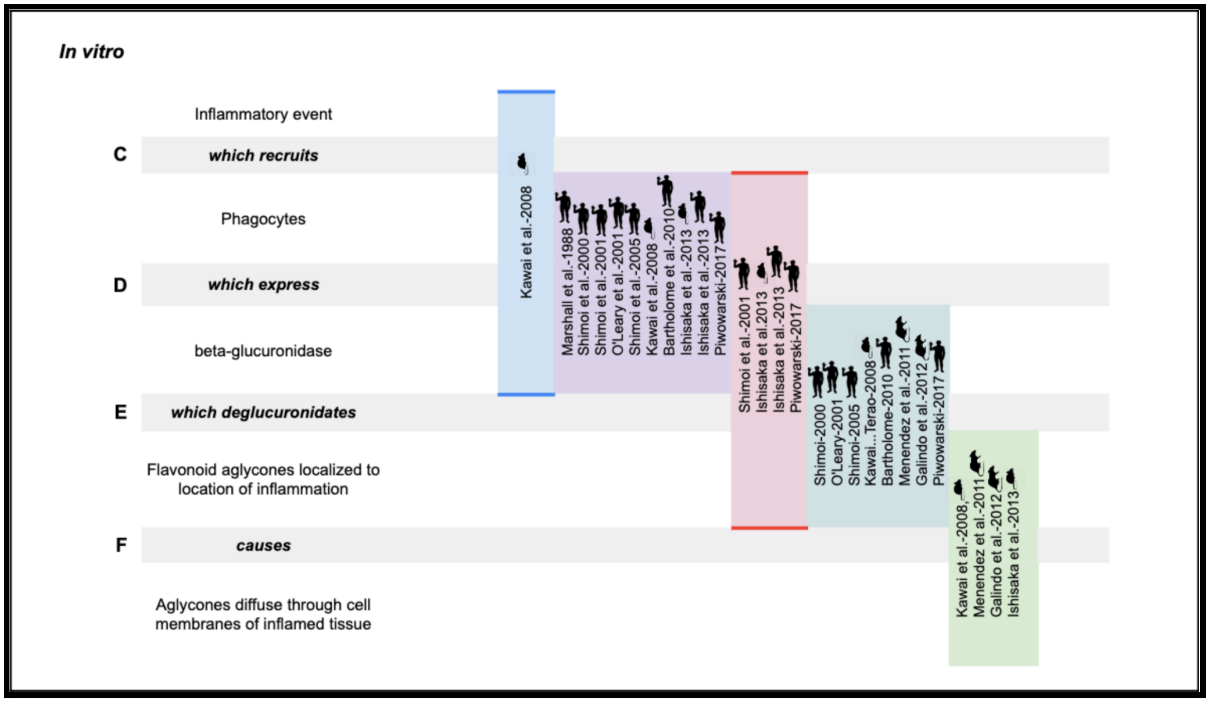
Stage B - Flavonoid aglycones are glucuronidated prior to arrival in the bloodstream
↓
Stage C - Neutrophils and macrophages are attracted to site(s) of inflammation
↓
Stage D - Beta-glucuronidase is expressed by neutrophils and macrophages
↓
Stage E - Serum flavonoid glucuronides are deglucuronidated ('deconjugated') by β-glucuronidase at site of inflammation
↓
Stage F - Flavonoid aglycones diffuse through cell membrane

Pathology-selective activation of glucuronide drugs by beta-glucuronidase has been widely understood and exploited in the context of anti-tumor agents.[49] However, it is the "deconjugation in inflammation hypothesis" that was developed and verified progressively over the period 2001-2019 across several polyphenols in vitro, in animal models, and in humans under inflammation conditions[65,67–78]. Steps of the pathway were verified across the polyphenols luteolin, quercetin, daidzein, and kaempferol, as well as the ellagic acid metabolites urolithin A, iso-urolithin A, and the single-phenol urolithin B. We propose standardizing the mechanism's naming to the technical term 'deglucuronidation-through-inflammation' or DTI. The 'Shimoi pathway' may also serve as a convenient shorthand that recognizes the lead researcher to first propose and study this mechanism with specific attention to inflammation with polyphenols (by way of luteolin).

6. Validation of deglucuronidation-through-inflammation

Demonstration of the evidence generated through the deglucuronidation-through-inflammation body of work[65,67–78], is provided against the model's labeled stages C-F in Figure 2.

Figure 2(A) - Deconjugation-through-inflammation literature basis table - in vitro support:



C - Phagocytes (whether neutrophils, macrophages or both) are recruited to the site of inflammation

D - Beta-glucuronidase is expressed by phagocytes (neutrophils, macrophages, or both)

CD - Beta-glucuronidase is expressed due to inflammation

E - Serum flavonoid glucuronides are deglucuronidated ('deconjugated') by beta-glucuronidase in situ at site of beta-glucuronidase expression

DE - Phagocytes deglucuronidate flavonoid glucuronides

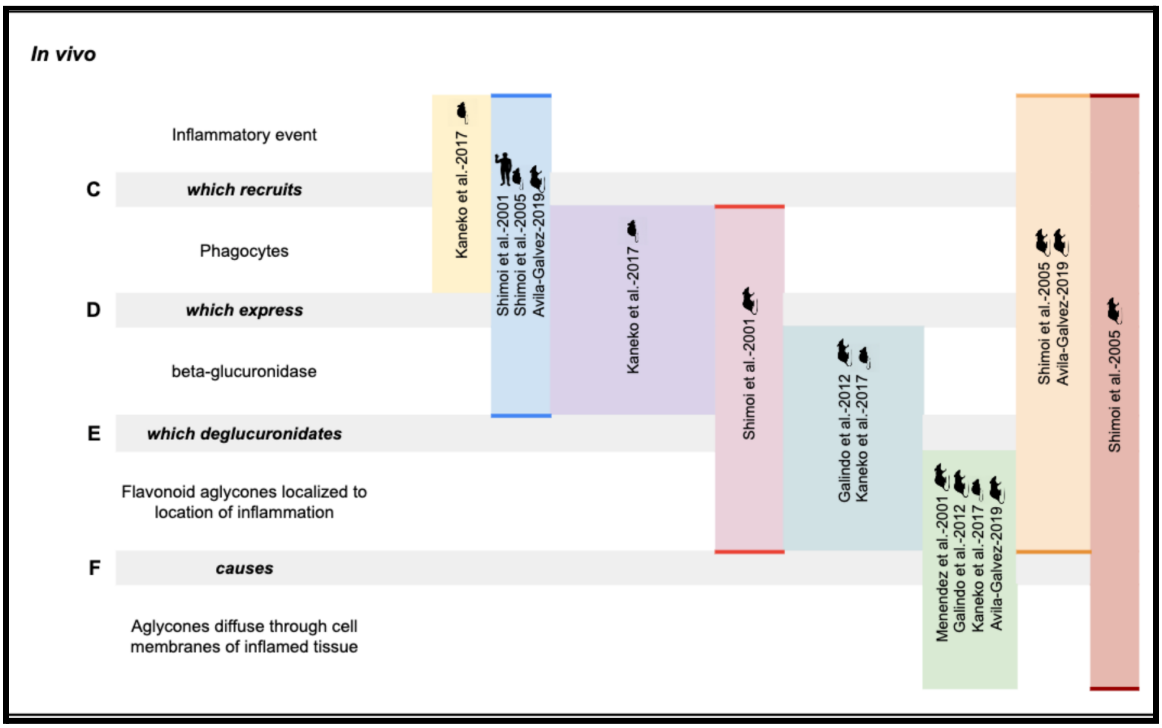
CDE - Inflammation causes deglucuronidation of flavonoid glucuronides

F - Flavonoid aglycones diffuse through any cell membrane / are uptaken by tissue / exhibit an effect that can only be mediated in-cell

Bold endcap cells designate end-to-end verification and exclude intermediary verification

mouse; rat; human

Figure 2(B) - Deconjugation-through-inflammation literature basis table - in vivo support:



C - Phagocytes (whether neutrophils, macrophages or both) are recruited to the site of inflammation

D - Beta-glucuronidase is expressed by phagocytes (neutrophils, macrophages, or both)

CD - Beta-glucuronidase is expressed due to inflammation

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CDE - Inflammation causes deglucuronidation of flavonoid glucuronides

F - Flavonoid aglycones diffuse through any cell membrane / are uptaken by tissue / exhibit an effect that can only be mediated in-cell

Bold endcap cells designate end-to-end verification and exclude intermediary verification

mouse; rat; human

While the deglucuronidation-through-inflammation hypothesis has been extensively reviewed by others[66,79–84], to our knowledge, this review is the first to unify the body of work into one cohesive, accessible evidentiary framework.

7. The promiscuous inhibition of polyphenols

Promiscuous inhibition poses two primary implications for medicinal chemistry assaying. The first is the non-specific binding of protein / enzyme targets themselves. The second is the disruption of assay integrity by inhibiting non-target enzymes used for assay readout. As it can be difficult to distinguish between these two, orthogonal assays are sometimes performed to verify a target binding interaction.

Promiscuity could take any of several forms. A promiscuous ligand could simply be highly conforming to a protein surface's geometry, with a high number of hydrogen donors & acceptors to more likely "stick" nonspecifically to any given protein site's own set of h-donors and h-acceptors. Another mechanism sees promiscuous inhibition take the form of colloidal aggregations.[85] In this mechanism, upon reaching a certain concentration, the ligand forms tightly-packed spherical aggregates with itself, even inside the cell.[86,87] Proteins and enzymes non-specifically bind to the surface of the aggregation and are inhibited in the process.[88] Often seen as a nuisance originally, it is now also seen as a source of opportunity in drug discovery as

well.[87,89,90] Deliberate study of aggregation in cell-based assays is a nascent sub-field[91] so cataloguing of non-specific aggregation among polyphenols in cells merits further investigation.

Figure 3 - Non-specific aggregation inhibition model

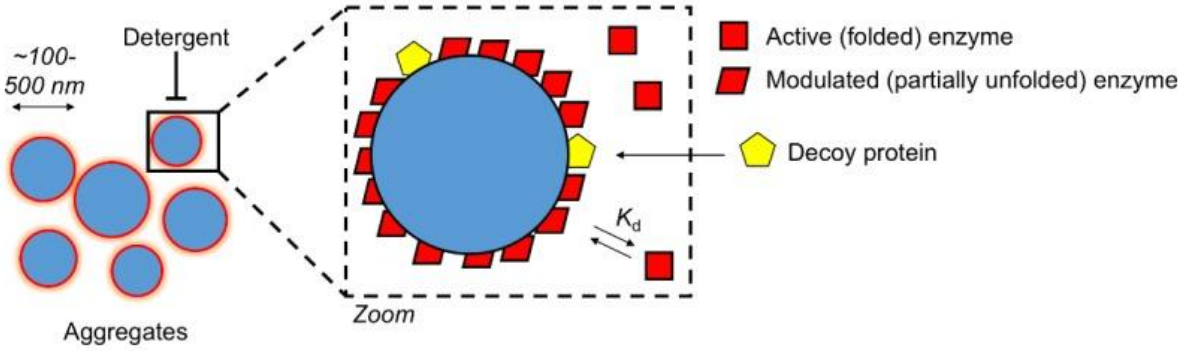

Figure reproduced from Auld et al. (2017)[88] under CC BY-NC-SA 3.0

Quercetin has earned a reputation as a promiscuous inhibitor[31,92–94] as well as having served as one of the first aggregators identified.[85,95,96] Myricetin[93,94,97–99] and tannic acid[93,94] are also promiscuous inhibitors and have both been further identified as aggregators.[94]

Of 123,844 assay records hosted by Pubchem and compiled by Gilberg et al. (2016)[100], their isolation of the most promiscuous 466 of them (99.6% percentile) contains 13 polyphenols based on our cheminformatic-oriented definition.

The catechol functional group, while not the exclusive province of polyphenols (and nor do polyphenols all contain catechol), certainly correlates with polyphenols. Bael and Holloway (2010), highlights catechol as a prominent PAINS functional group[101] even as Capuzzi et al. (2017) cautions against blind application of PAINS filters[102]. And yet Jasial et al. (2017) demonstrates that the catechol functional group is in the top ten percentile (9.5) of primary activity assays in Pubchem, and in the top seven percentile (6.9) of functional groups in Pubchem confirmatory assay activity.[103]

We reviewed the 437,257 compounds of Pubchem Bioassay contributions[93] compiled by Jasial, 2016[98] and filtered through compounds with greater than 40 confirmatory assays to yield 392,259 compounds. Against this list, we checked our list of 263 primarily natural products, curated prior to outlining the research agenda for the current work, and which contains polyphenol and non-polyphenols. The natural products list was timestamped and then cross-referenced against the filtered database yielding 82 compounds appearing in both datasets. The results were ranked for assay and target promiscuity and are presented in Table 2.

The cross-referenced results and ranking demonstrate that polyphenols are indeed promiscuously binding, with most ranking above the 95-percentile rank in this dataset. Single-phenol compounds from the same list yield a mean percentile of 72.5% and median percentile of 89.9%. The non-phenol (and non-cyclohexanol) natural compounds remaining in our list show a mean percentile of 28.8% and a median percentile of 0%.

The source list quantifies, yet can not be capable of distinguishing between, assay promiscuity and target promiscuity. But even as many assays depend on enzymes for their readouts, such as luciferase,[104] a broad interference with assays will also contribute to a compound's general promiscuity profile.

Table 2 - Promiscuity ranking of several polyphenols in 437,257 Pubchem Bioassays

Polyphenol? (chem-informatic def)	Compound	Pubchem CID	Submitted Confirm-atory Assays count	Assay hits count in Confirma-tory Assays	Assay Promiscuity (APC)	APC Percentile Ranking	Target hits count in Confirm-atory Assays	Target Promiscuity (TPC)	TPC Percentile Ranking
Y	Genistein	5280961	218	33	15.1%	98.9%	24	11.0%	98.6%
Y	Resveratrol	445154	206	39	18.9%	99.4%	29	14.1%	99.2%
Y	Daidzein	5281708	200	19	9.5%	97.2%	17	8.5%	97.6%
Y	Apigenin	5280443	188	33	17.6%	99.3%	27	14.4%	99.3%
Y	Myricetin	5281672	182	60	33.0%	99.9%	44	24.2%	99.8%
Y	(-)-Epicatechin	72276	176	10	5.7%	92.5%	10	5.7%	94.4%
Y	Rhein	10168	164	27	16.5%	99.1%	23	14.0%	99.2%
Y	Isoliquiritigenin	638278	159	19	11.9%	98.2%	15	9.4%	98.1%
Y	Ellagic Acid	5281855	154	38	24.7%	99.7%	32	20.8%	99.7%
Y	Kaempferol	5280863	143	17	11.9%	98.2%	14	9.8%	98.2%
Y	Quercetin	5280343	142	38	26.8%	99.8%	34	23.9%	99.8%
Y	Chrysophanol	10208	139	7	5.0%	91.2%	6	4.3%	91.1%
Y	Curcumin	969516	137	34	24.8%	99.7%	28	20.4%	99.7%
Y	Naringin	442428	129	3	2.3%	77.5%	2	1.6%	70.8%
Y	Naringenin	932	128	1	0.8%	54.2%	1	0.8%	54.2%
Y	Luteolin	5280445	127	34	26.8%	99.8%	29	22.8%	99.8%
Y	Hesperetin	72281	115	5	4.3%	88.8%	5	4.3%	91.1%
Y	Aloe-emodin	10207	112	3	2.7%	80.1%	3	2.7%	83.5%
Y	Diosmin	5281613	111	5	4.5%	89.5%	5	4.5%	91.7%
Y	Amentoflavone	5281600	111	14	12.6%	98.4%	11	9.9%	98.3%
Y	Vicenin-2	442664	107	0	0.0%	0.0%	0	0.0%	0.0%
Y	Magnolol	72300	104	7	6.7%	94.5%	5	4.8%	92.6%
Y	Cirsimaritin	188323	101	3	3.0%	82.6%	3	3.0%	85.8%
Y	Catechin	9064	101	7	6.9%	94.9%	6	5.9%	94.8%
Y	Hesperidin	10621	98	2	2.0%	74.4%	2	2.0%	77.0%
Y	Chrysoeriol	5280666	97	4	4.1%	87.9%	4	4.1%	90.3%
Y	Rutin	5280805	89	6	6.7%	94.5%	6	6.7%	96.0%
Y	(-)-EGCG	65064	84	13	15.5%	99.0%	13	15.5%	99.4%
Y	Kaempferol-3-O-Glucoside	5282102	80	4	5.0%	91.2%	3	3.8%	88.7%
Y	Isovitexin	162350	79	0	0.0%	0.0%	0	0.0%	0.0%
Y	Puerarin	5281807	76	0	0.0%	0.0%	0	0.0%	0.0%
Y	Luteolin-7-O-Glucoside	5280637	73	7	9.6%	97.2%	7	9.6%	98.2%
Y	Acteoside	5281800	62	7	11.3%	98.0%	5	8.1%	97.3%
Y	Isoquercitrin	5280804	48	9	18.8%	99.4%	8	16.7%	99.5%
Y	Tannic Acid	16129778	48	18	37.5%	99.9%	15	31.3%	99.9%
Y	Rhamnetin	5281691	44	4	9.1%	96.9%	4	9.1%	97.9%
Y	Gossypetin	5280647	42	9	21.4%	99.6%	8	19.0%	99.7%
Y	Rosmarinic Acid	5281792	41	13	31.7%	99.9%	12	29.3%	99.9%
Y	Gallocatechin Gallate	1287	41	11	26.8%	99.8%	9	22.0%	99.8%

Results for the single-phenol and non-polyphenol natural products from our list can be found in the supplemental materials and Zenodo.[105] Notably, diosmetin has seen too few bioassays contributed to Pubchem to ascertain its promiscuity statistically. The compounds from this dataset that broadly contain any of the flavonol/flavanone/isolflavonid/flavone/flavan backbone scaffolds irrespective of phenol or hydroxyl count, also show strong correlation with promiscuity. Indeed, superficial inspection suggests that a Quideau definition that 1) takes into account biosynthesis provenance and 2) allows for a single phenol ring may show the highest correlation with promiscuity of all categorizations considered here.

8. Therapeutic role of a promiscuous binder?

The final step of a putative polyphenol deglucuronidation-based antiviral mechanism requires that a promiscuous-binding compound once inside a virus-infected human cell will arrest viable virion production.

Inhibitory mechanisms of viral replication could be due to direct inhibition of viral proteins and enzymes, or by slowing ordinary cellular metabolic mechanisms such as respiration, translation, transcription as co-opted by the infecting virus. In one case, that of fisetin applied to Dengue fever,[106] fisetin showed no direct activity against DENV virions outside the cell yet effectively inhibited replication in-cell. The authors suggest it could be due to forming complexes with RNA or inhibition of RNA polymerases. While inhibition of the dengue RdRp would represent a virus-specific inhibition, it remains intriguing to consider that the replication inhibition could also be due to non-specific inhibition in a weakened cell.

Table 3 - proposed deglucuronidation-based antiviral mechanism

Stage A - Infection by any of several virus species induces inflammation.
↓
Stage B - Flavonoid aglycones are glucuronidated prior to arrival in the bloodstream
↓
Stage C - Neutrophils and macrophages are attracted to site(s) of inflammation
↓
Stage D - Beta-glucuronidase is expressed by neutrophils and macrophages
↓
Stage E - Serum flavonoid glucuronides are deglucuronidated (‘deconjugated’) by β-glucuronidase at site of inflammation
↓
Stage F - Flavonoid aglycones diffuse through cell membrane
↓
Stage G - Flavonoid aglycones cause non-specific inhibition within the cell - interfering with both ordinary cellular processes and the etiological source of inflammation (such as viral replication)

Table 3 is illustrated graphically in Figure 4 - by way of one of the most studied flavonoids in the pharmacokinetic literature, quercetin.

Figure 4: Model of mechanism-of-action for deconjugated quercetin during viral infection

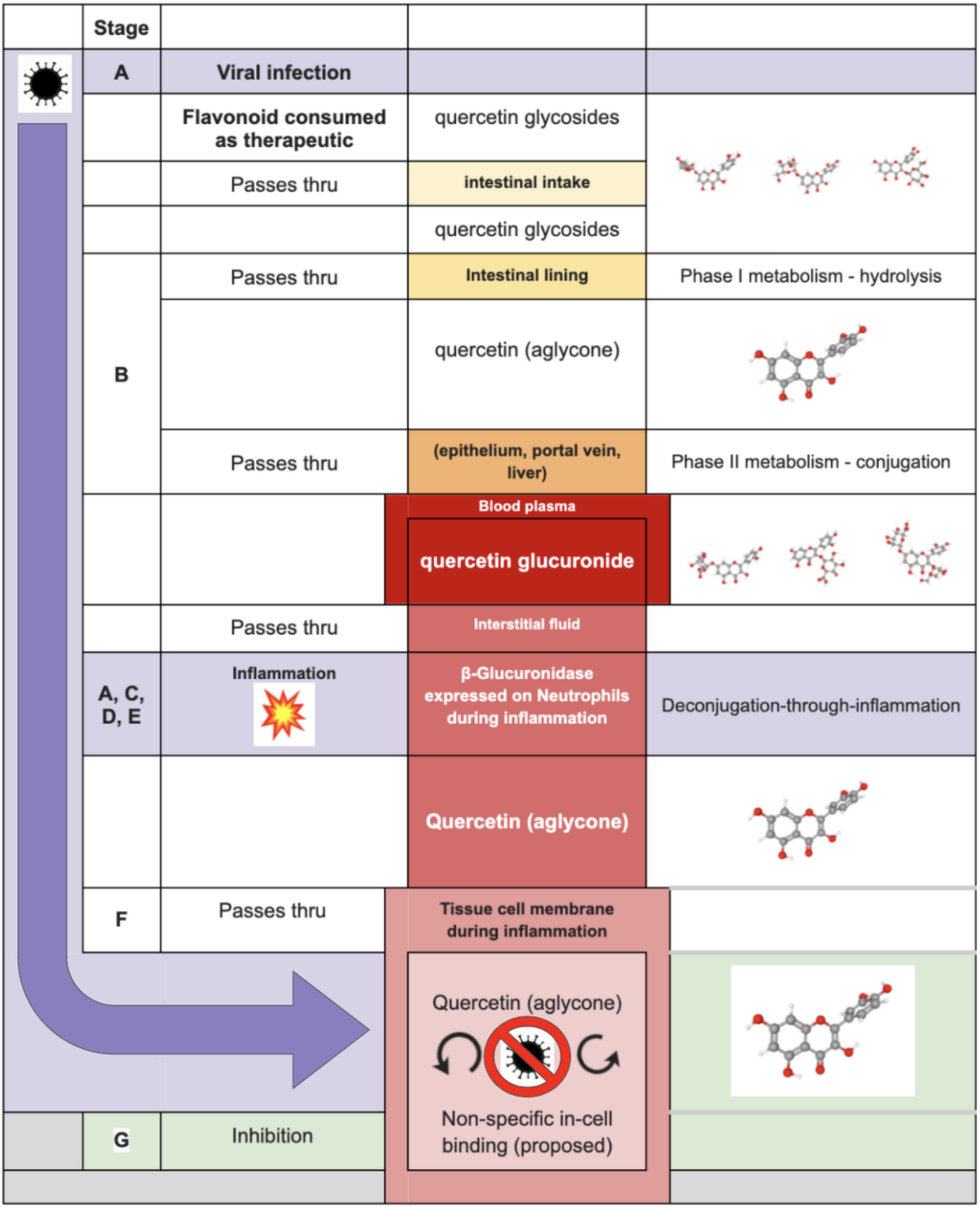

Figure 4: quercetin,[107] metabolites,[108–112] and mechanism for entry into intracellular compartment (adapted from Perez-Vizcaino et al 2013[66]).

9. Application of DTI to antiviral assaying and clinical trialing

Given that many forms of viral infections are known to induce inflammation, it would be a logical extension to study whether consumption of certain flavonoids of sufficient quantity and in bioavailable forms could serve to reduce the rate of viral replication in the early stages of viral infection. The mechanism of action could be by inhibition of viral entry to cells, direct inhibitory action on viral enzymes in-cell, or non-specific promiscuous disruption of the co-opted metabolism of infected cells.

The literature provides early in vitro evidence of achievable inhibition by phenolic flavonoids spanning across Dengue virus, Influenza-A virus (IAV), Chikungunya virus, Foot-and-mouth disease virus (FMDV), Japaneses Encephalitus Virus (JEV) and SARS-CoV-2.

Table 4 - in vitro evidence of inhibition by phenols and polyphenols

	DENV	FMDV	Influenza-A	JEV	CHIKV	SARS-CoV-2
Luteolin		9.7-10.0μM[113]		15.9μM[114]		
Isoginkgetin		1.9-2.0μM[113]				
Quercetin	95.6-118μM[115]		8.9-25.8μM[116]			18.2μM[117]
Baicalin*			97-235μM[22]	0.8-3.2μM[118]	7.0μM[119]	2.9μM[120]
Curcumin	14.0μM[121]		0.5-3.8μM[122,123]		3.9μM[124]	0.4-38μM[117,125,126]
Fisetin	150μM[106]				29.5μM[119]	
Quercetagetin					43.5μM[119]	
Naringenin	18-180μM[106]					< 35μM[127]

* one phenol only

Much care must be applied in interpreting in vitro viral replication inhibition results. Where an IC50 value is defined against a measure of viral RNA copies/mL, then qRT-PCR will show a difference of a single unit of Ct. For comparison, SARS-CoV-2 infection typically presents a Ct range between 10 and 40 for acute infection vs non-detectable viral load, respectively.[36] However, in vitro and in vivo Ct values are not directly comparable, as in vitro reduction of viral replication may exhibit nonlinear effects at the in vivo scale, especially when the effects of the innate and specific immune system are considered. Where due analysis of toxicity allows, a higher IC value can be targeted, such as IC90 or even IC99.[128]

Viral inhibitory assays typically report the Selectivity Index (SI), defined as the ratio of the cytotoxicity (CC50) to the inhibitory concentration (IC50). A SI < 1 means that the ligand's cytotoxicity to cells occurs at a lower concentration than its inhibition of the target. Selectivity indices of 5 or greater are preferred. Ligand candidates suffering from lower selectivity indices may be excluded from further investigation. However, the deglucuronidation-through-inflammation mechanism would suggest that dismissing polyphenol ligands with a low selectivity index could be overly conservative. Given that most polyphenols circulate in plasma as glucuronides, and are only deglucuronidated to their aglycone form locally to the site of inflammation, a low

selectivity index may be acceptable and even preferable. This of course will depend on how efficiently the deglucuronidation process discriminates between the localities of healthy and infected cells that induce the inflammatory process.

Given the specificity that deglucuronidation-through-inflammation affords, and the putative validity of aggregation-based nonspecific binding mechanisms,[86] the standard practice of applying aggregate-dissociating detergents such as Triton X-100 is called into question for antiviral assays of phenols that are known or expected to act through the DTI mechanism. A revisiting of relevant results of in vitro assays in the literature where such a detergent was applied would be appropriate. However, such a modification to laboratory practice should be considered carefully as the tendency for an aggregation to bind assay-specific enzymes could still benefit from detergent application.

Further laboratory and clinical investigation of demonstrably promiscuous-binding polyphenols in in vitro viral infection culture and in vivo will continue to be valuable. Results of recent clinical trials of a polyphenol, quercetin, even while preliminary, certainly do not discourage further investigation.[129,130] Attention would be particularly appropriate against those viruses that are known to induce inflammatory responses such as influenza A (IFV-A), dengue (DENV), chikungunya (CHIKV), and coronavirus (SARS-CoV-2).

10. Additional observations on antivirals trialing of polyphenols

While it is important to maintain ligand concentration at the target for a period sufficient to exert the relevant mechanism of action, it is worth noting that this period can be extremely short. Although not a polyphenol, artemisinin enjoys enormous efficacy against the malaria parasite *P. falciparum* with a T_{max} at less than 2 hours and a half-life of 2-5 hours.[131,132] Also, the dosage is of utmost importance. Following due analysis of toxicity, protease inhibitors can target a C_{min} dose (minimum concentration between consecutive doses) of many multiples of the IC50 value[133] to achieve faster viral clearance. Indirect antiviral effects of certain polyphenols may also be possible, such as non-specific upregulation of immunosurveillance, as well as modulation of specific immune cells.[134–136]

Also, as promiscuous binders, due attention should be applied to inhibition of liver enzymes for drug-drug interactions[44], especially of drugs that study subjects might concomitantly consume for the same or unrelated conditions. For example, among the polyphenols studied are those known to bind to CYP1A2, CYP3A4 and OATP1A2, the latter giving rise to the famous “grapefruit effect”.[137] Conversely, this P450 or other liver enzyme inhibition may be advantageous to increase serum concentrations of verified pharmaceuticals, such as fluvoxamine, in context of combination therapy (J. Duke, personal communication, 28 Nov 2009) for superior joint bioavailability.

Finally, as a given polyphenol can demonstrate differing bioavailabilities between different dosage forms,[57] consideration should also be given to oral delivery type, such as aqueous, softgel, dry tablet form, and degree of micronization. Further, owing to strong bioavailability and/or release rate, delivery in the form of original plant matter while controlling for phytochemical content should also be considered.[72,138,139]

11. Evolutionary Role

A BLAST search demonstrates that the gene coding for β-glucuronidase (GUSB, and uidA in bacteria[140]) is extensively common across the animal kingdom (data not shown). Its homolog β-galactosidase (40% identity) is also commonly expressed in bacteria (data not shown). β-glucuronidase has several documented purposes[140]. It targets glucuronic acid in the gut,[141,142] and is associated with the degradation of glucuronate-containing glycosaminoglycan.[143] But its extensive expression on, and release from, neutrophils

attracted in response to inflammatory signals is a mechanism whose genetic etiology and species prevalence will require further work to elucidate.

Given the long history of herbivory in animals (and associated polyphenolic compound ingestion), and the high prevalence frequency of the β-glucuronidase-coding gene GUSB across vertebrates, this mechanism could be a long-ago evolved broad response under selection pressure of viral pathologies in ancestral species. It would be worthwhile for future investigators to probe the genetic basis for neutrophilic β-glucuronidase expression and its orthology across vertebrate species in order to better localize how this response evolved.

12. Conclusion

In this paper, we add to the body of evidence in the literature that polyphenols are a frequent-binding class of chemicals produced by plants. We show that the pharmacology of polyphenols may allow for viral infection-fighting potential due to the human body's inflammatory response and provide conjecture as to the evolutionary basis for a putative inflammation-induced antiviral function. Future work could include quantifying the effect of in vitro antiviral studies under inflammation with neutrophils present for such viral targets as SARS-CoV-2, IAV/IBV, CHIKV, DENV and others.

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Competing Interests statement

RS leads research & development for EMSKE Phytochem, focusing on efficacy verification of phytochemical constituents. He also leads a venture in the agricultural advisory industry.
KS is CSO of Health, Education & Research and an advisor and consultant for the natural products industry.

The authors declare no competing interest.

Supplementary Materials

The supplementary materials are posted to Zenodo at https://zenodo.org/record/5776867# .

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