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Meta-analysis of flavonoids with antiviral potential against coronavirus

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SUMMARY

Preliminary studies which may be of significance for research against coronaviruses, including SARS-CoV-2, which has caused an epidemic in China, are presented. An analysis was made of publicly available data that contain information about important metabolites neutralizing coronaviruses. Preliminary studies show that especially Ficus, barley, thistle and sundew should be additionally tested with the aim of producing medicines for coronavirus.

Key words: coronavirus, meta-analysis, flavonoids, rhoifolin, pectolinarin, herbacetin, ANOVA, Tukey's HSD test, barley, Ficus, thistle, sundew, wheat, soybean, *Brachypodium distachyon*

1. Introduction

In December 2019, Wuhan, Hubei province, China, became the center of an outbreak of pneumonia of unknown cause; see Wang et al. (2020). The emergence of a SARS-like coronavirus (SARS-CoV-2) and its rapid spread through many countries have been labeled as an international emergency. The antiviral activity of some metabolites (from the group known as flavonoids) against CoVs is presumed to be caused directly by inhibition of 3C-like protease (3CLpro). In Jo et al. (2020) a flavonoid library was used to systematically probe inhibitory compounds against SARS-CoV 3CLpro. Rhoifolin, pectolinarin and herbacetin were found to efficiently block the enzymatic activity of SARS-CoV 3CLpro. Based on the systematic analysis, the three flavonoids were suggested to serve as templates to design functionally improved inhibitors. Simplifying, all coronaviruses produce proteins that have a negative effect on our immune system. Jo et al. (2020)

found that especially three natural plant products, rhoifolin, pectolinarin and herbacetin, block the activity of these proteins. They attach to active sites of proteins and thereby inactivate them, thus neutralizing the viruses. All three compounds belong to the group of phenolic structures with flavonoid skeleton, which have many functions in plants; see Decker (1997). The bioactivity of flavonoids may be related to their ability to chelate metals, inhibit lipoxygenase and remove free radicals. In addition, they can act as free radical scavengers and terminate radical chain reactions that occur during the oxidation of triglycerides; see Das et al. (1990).

In this publication we focus on the best source of metabolites with antiviral potential against coronavirus, since this problem has not been previously considered in the literature and may be helpful in the fight against SARS-CoV-2.

2. Materials and methods

We have conducted bioinformatic meta-analyses of the world's largest publicly available scientific repository databases: Metabolights (https://www.ebi.ac.uk/metabolights/) and Prime (http://prime.psc.riken.jp/menta.cgi/prime/drop_index), as well as scientific publications in which the described substances have been identified. These datasets contain publicly available metabolic data matrices deposited by scientists and derived from high-throughput analytical methods, including liquid chromatography coupled with mass spectrometry. The databases were searched with the keywords *rhoifolin*, *pectolinarin*, *herbacetin*. A set of experiments was identified for each of the searched phrases. Our analysis concerned experiments in which these pro-health phytochemicals were reported. We found data matrices for plants known for their high phytochemical activity: barley, wheat and *Brachypodium distachyon* from the grass family, as well as thistle, sundew, soybean and Ficus. These plants were selected as plants having publicly available data that can be used for statistical analysis. Each of these plants has exactly one of the three considered compounds.

All data sets concerned different types of plant treatment and experimental conditions. Therefore only samples subject to control conditions were selected for analysis. Information about the considered data sets, with numbers of samples subject to control conditions and known metabolites, is given in Table 1.

Table 1. Basic information on the analyzed data sets

Plant	Variety/ Species/ RILs	Part of plant	No of samples for control conditions	No of described metabolite	Source of data set
Sundew	*Drosera peltata*	Leaf	4	11	Braunberger et al. (2012)
Thistle	*Cirsium japonicum*	Leaf	15	10	Ge et al. (2013)
Ficus	*F. deltoidea*	Leaf	100	387	MTBLS756 https://www.ebi.ac.uk/metabolights/MTBLS756
Barley	CamB1	Leaf	23	104	MTBLS52 https://www.ebi.ac.uk/metabolights/MTBLS52
	Georgia	Leaf	20	104	
	Harmal	Leaf	18	104	
	Lubuski	Leaf	19	104	
	Maresi	Leaf	21	104	
	MDingo	Leaf	16	104	
	Morex	Leaf	21	104	
	Sebastian	Leaf	21	104	
	Stratus	Leaf	22	104	
Wheat	Chinese Spring	Seed	9	371	DM0020 http://prime.psc.riken.jp/menta.cgi/prime/drop_index
	Chinese Spring	Leaf	9	371	
Brachypodium	Line Bd21	Seed	9	517	DM0020 http://prime.psc.riken.jp/menta.cgi/prime/drop_index
	Line Bd21	Leaf	9	517	
	Line Bd3-1	Seed	9	517	
	Line Bd3-1	Leaf	9	517	
Soybean	recombinant inbred lines of *G. max* and *G. soja*	Seed	279	48	DM0011 http://prime.psc.riken.jp/menta.cgi/prime/drop_index

3. Results and discussion

In the case of sundew, the analyzed trait was the percentage of herbacetin content in the sum of all known from literature phenolic metabolite contents in each sample. Then the mean of the trait for all samples was calculated. In the case of Ficus, thistle, barley, wheat, *B. distachyon* and soybean, the analyzed trait is the percentage content of the considered metabolite in the sum of all known from literature metabolite contents in each sample. Statistical analysis for barley was performed in R. All visualizations were prepared in R. For barley, two-way ANOVA was performed for two factors:

variety and time point (plant growth stage); see Table 2. Differences between means were significant only for the variety factor, with a p-value equal to 0.0106. Additional one-way ANOVA was performed for the variety factor; see Table 3. Means for the content of rhoifolin were greatest for Stratus and Morex. Differences between mean values for varieties were tested for significance using Tukey's honest significant difference test (at the 5% level), giving the results shown in Table 3.

Table 2. Results of two-way ANOVA for variety and time point, where T1, T2, T3, T4, T5, T6 are different development phases, the first three referring to seedlings at the three-leaf stage and the last three to plants at the flag leaf stage

	Mean					
Variety	T1	T2	T3	T4	T5	T6
CAMB1	0.3219	0.2015	0.1613	0.2377	0.2722	0.1952
GEORGIA	0.2314	0.1651	0.1956	0.1676	0.1378	0.1550
HARMAL	0.3683	0.1303	0.0000	0.2355	0.2598	0.3216
LUBUSKI	0.0000	0.1681	0.0939	0.1969	0.1822	0.2316
MARESI	0.2104	0.1491	0.1004	0.1873	0.1285	0.1367
MDINGO	0.4600	0.1103	0.1623	0.3004	0.1534	0.1976
MOREX	0.6093	0.2986	0.2508	0.2771	0.1950	0.1826
SEBASTIAN	0.1476	0.1533	0.1433	0.1933	0.1545	0.2185
STRATUS	0.3863	0.2219	0.6737	0.3538	0.1948	0.3221
	Standard deviation					
Variety	T1	T2	T3	T4	T5	T6
CAMB1	0.2146	0.2161	0.1186	0.0800	0.3190	0.1274
GEORGIA	0.0000	0.0523	0.1094	0.0264	0.0591	0.0785
HARMAL	0.2265	0.0589	0.0000	0.0364	0.1271	0.2021
LUBUSKI	0.0000	0.1374	0.0341	0.0273	0.0321	0.1034
MARESI	0.1413	0.0410	0.0050	0.0345	0.0270	0.0286
MDINGO	0.4222	0.0058	0.0000	0.1349	0.0698	0.0000
MOREX	0.4219	0.1612	0.0895	0.0509	0.0325	0.0283
SEBASTIAN	0.0638	0.0412	0.0350	0.0174	0.0351	0.1259
STRATUS	0.3452	0.1352	0.9756	0.0577	0.0633	0.0911

Table 3. Results of one-way ANOVA and Tukey's honest significant difference test

Variety	Mean of variety	SD of variety	HSD test
CAMB1	0.2277	0.1793	ab
GEORGIA	0.1660	0.0623	b
HARMAL	0.2514	0.1422	ab
LUBUSKI	0.1715	0.0833	ab
MARESI	0.1542	0.0620	b
MDINGO	0.2211	0.1748	ab
MOREX	0.2990	0.2050	ab
SEBASTIAN	0.1677	0.0580	b
STRATUS	0.3592	0.4238	a

Herbacetin is a rare phytochemical with proven anticancer activity; see Kim et al. (2016). It was reported in sundew (*Drosera peltata*) in Braunberger et al. (2013) and the data are used here for statistical analysis, which shows that herbacetin constitutes about 1.38% of all phenolic compounds in *Drosera peltata*. In medicine and herbal medicine, the analgesic and expectorant properties of sundew extracts have been used for centuries; see Czygan (2004). Herbal extract from this insectivorous plant contains a variety of active natural products.

A second rare metabolite called pectolinarin has been identified in thistle (*Cirsium japonicum*). This plant is closely related to teasel and cabbage thistle. In Chinese folk medicine, it is widely used as an antihemorrhagic, antihypertensive and diuretic agent as well as an antiviral agent; see Czygan (2004). According to the data from Ge et al. (2013), which are analyzed here, pectolinarin constitutes as much as 53.89% of known natural compounds in leaves of thistle (*Cirsium japonicum*). Moreover, it was reported by Cho et al. (2016) that in *C. japonicum* leaves pectolinarin constitutes 0.1% of the extract obtained in the experiment and pectolinarin content in leaves of *Cirsium* species was higher than that in the root and pappus. In addition, the leaves of *C. chlorolepis*, have a higher content of this compound than the leaves of *C. japonicum* and other species: *C. chanroenicum*, *C. nipponicum* and *C. setidens* (see Figure 1).

Rhoifolin is the most widespread of the studied metabolites. It has been identified in barley, wheat, soybeans, Ficus, and *B. distachyon*, which is phylogenetically close to barley and wheat (Afzan et al. (2019), Piasecka et al. (2017), Onda et al. (2015), Sawada et al. (2016)). Preclinical studies have shown that rhoifolin possesses a variety of significant biological activities in-

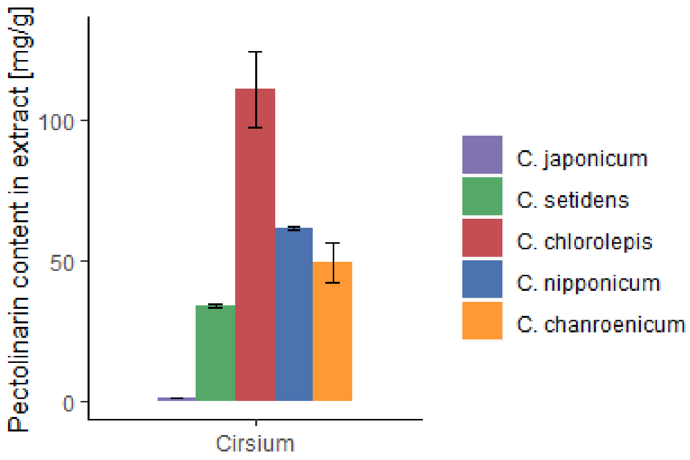

Figure 1. Means and standard deviations for the content of pectolinarin in the leaf extract of five *Cirsium* species, expressed in mg/g

cluding antioxidant, anti-inflammatory, antimicrobial, hepatoprotective and anticancer effects; see Refaat et al. (2018).

Barley is another plant rich in rhoifolin. Research on the leaves of barley was carried out in Piasecka et al. (2017). In a pilot experiment, nine varieties of barley were considered at different development phases, designated as time points (T1, T2, T3, T4, T5, T6) in Figure 2. The first three time points refer to barley seedlings at the three-leaf stage (16 days after sowing) and the last three to plants at flag leaf. We find that the highest content of rhoifolin occurs in specific stages of development of the barley varieties Stratus and Morex (see Figure 2 and Figure 3). The dendrogram shows three groups of varieties, consisting of: a) Stratus (this underlines how this variety differs from the others); b) CamB1, MDingo, Harmal, Morex; c) Georgia, Maresi, Sebastian, Lubuski. A blue color in the heatmap indicates variants with the greatest percentage of rhoifolin. Various medicinal products have already been produced from barley. Our preliminary research indicates that it will be beneficial to study barley phytochemicals and to compare it with the other plants listed here for further research on coronavirus medicines.

The databases show the presence of rhoifolin in the experiments on wheat leaves and grains and two varieties of *Brachypodium distachyon* presented in Onda et al. (2015), and in the soybean recombinant inbred lines

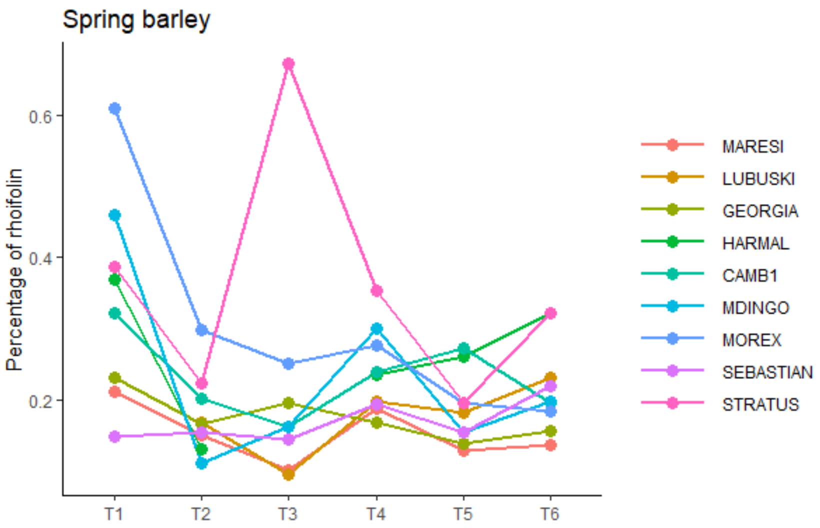

Figure 2. Mean values for the percentage of rhoifolin in the sum of all known metabolites, for specific cultivars and developmental stages of the plant, in nine spring barley varieties. Stratus had the highest percentage at time T3, and Morex at time T1

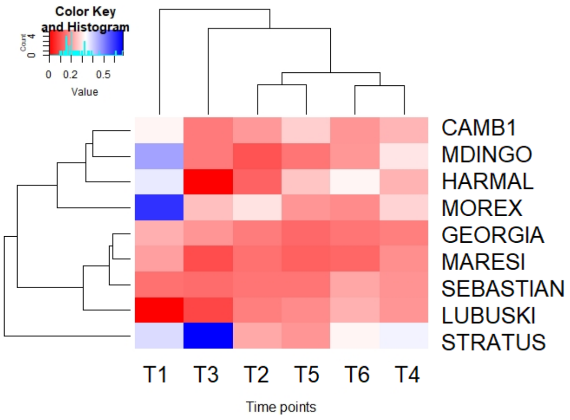

Figure 3. Heatmap with mean values for the percentage of rhoifolin for each barley variety and time point. Blue represents the greatest values. Dendrograms show groups of varieties and time points. The Stratus variety clearly stands out

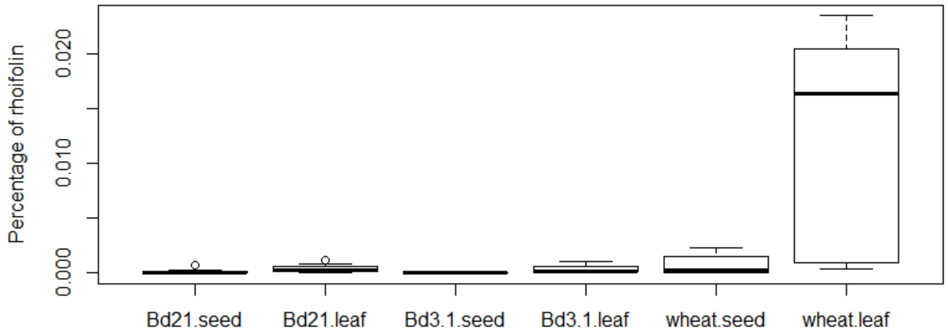

Figure 4. Boxplots showing means, quartiles and range of percentage of rhoifolin in the sum of all known metabolites for: two lines of *Brachypodium* (Bd21 and Bd3-1) and wheat (Chinese Spring), in leaves and roots, separately

of *G. max* and *G. soja* considered in Sawada et al. (2016). *B. distachyon*, commonly called purple false brome or stiff brome, is a plant closely related to wheat and barley, but it has not been so extensively studied as its agronomic relatives. Due to the similarity of metabolomic profiles between *B. distachyon* and barley and wheat, it is worth considering as a potential source of health-promoting substances. The third highest percentage of rhoifolin content in the sum of all known metabolites in the studied plants was recorded in wheat leaves in Chinese Spring, considered in Onda et al. (2015), and deserving of further comprehensive research conducted in standardized experimental conditions. Although wheat extracts have not been tested for antiviral properties, fermented wheat germ extract is a licensed nutrient for cancer patients as well as an antiproliferative and antimetabolic factor; see Otto et al. (2016). In Figure 4 we present boxplots for an experiment carried out jointly for *B. distachyon* and wheat. Leaves of wheat have a higher percentage rhoifolin content than the other considered variants.

4. Conclusions

All of the tested plants should be considered in research on the development of drugs against coronaviruses, including SARS-Cov-2, due to the

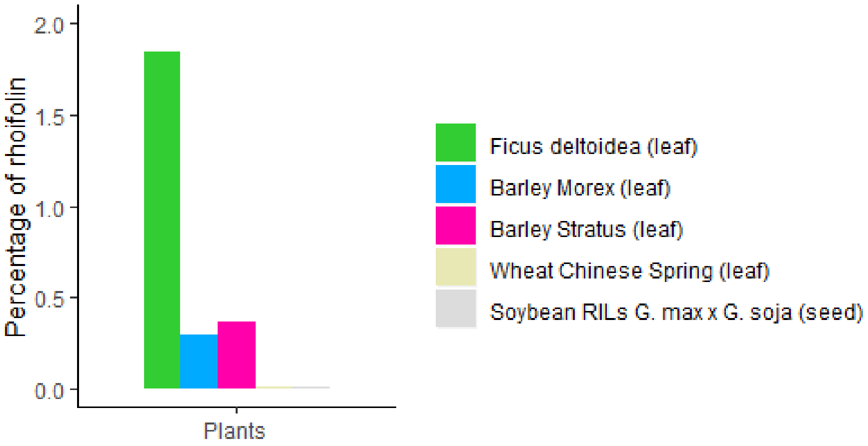

Figure 5. Mean values for the percentage of rhoifolin for the highest results in each analyzed group of plants, calculated among all known metabolites for each variant.

high content of rhoifolin, in particular in the leaves of Ficus and barley (see Figure 5), but also the antiviral metabolites in sundew and thistle. Further research on wheat, *B. distachyon* and soybeans may also be beneficial. A next step to gain more knowledge about the problem would be to perform experiments with equivalent conditions for the considered plants, especially to verify whether in repeated experiments the barley varieties Stratus and Morex will produce similar results to those reported here.

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