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# Cistus × Incanus L. Pandalis and Its Broad Antiviral Properties

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**Abstract:** Derived from Cistus x incanus L. Pandalis, the extract Cystus Pandalis® has been subject to several examinations concerning its antiviral effect. Its effectiveness has been proven in all examinations in-vitro and in-vivo. In physiological dosages, the Infection of cell cultures by applied viruses was completely prevented in-vitro without the cells being affected by Cystus Pandalis® extract. Likewise, the application of Cystus Pandalis® extract in-vivo showed that it prevented the infection of subjects, in contrast to subjects of the control group living in similar life situations. The effect is based presumebly on the specific highly concentrated polyphenols typical to the variety in the used extract. Its dense concentration of polymeric polyphenols (over 90%) in contrast to its relativeley low content of monomeric polyphenols (about 2%) seems to be key to this specific extract. So it blocks the cellular attachment sites and inhibits the virus from entering. As it is for SARS-CoV-2, it seems plausible that the extract encases surface proteins (in this case the Spike-Protein) to prevent the virus from attaching to the human cell and, therefore, avert infection. Cystus Pandalis® extract is available as lozenges or mouthrinse in form of a decoction. Prophylactic use during infectious waves and viral epidemics/pandemics at any stage is easily feasible. The extract is well-tolerated and has caused no known sideeffects. So far, no viral resistances or the development thereof have been detected. In Addition, no contraindications or interactions with other drugs are known to date. Cystus Pandalis® extract is freely available and can be applied regardless of age or sex.

**Keywords:** Cystus Pandalis®, Virus Infection, SARS-CoV-2, Antiviral, Cistus Incanus, Pathogens

## 1. Introduction

Viruses came into place a billion years after our solarsystem was born. Science's stance for now states: Earth is about 4.6 billion years old. 3.8 billion years ago the first biomolecule came into being. These molecules were able to multiply by attaching new molecules onto themselves creating copies of their own. [1] Sometimes though, mutations emerged through transcription errors and thrusted the development of life on Earth foward. These mutations were essential for evolution.

Today, three theses circulate regarding the origin of viruses. The first thesis claims viruses are genes that went autonomous, respectively are chromosomeparts no longer bound to the host. Another thesis says viruses were once bacteria having lost parts of its genetic building blocks. Thirdly and the most popular thesis argues that the first ever cell in the history of Earth had already been infected by viruses and that viruses have therefore been part of every evolutionary development, forming newly varieties forever.

The first ever viruses had developed long bevor all human or animal life. Over time and gradually, those old viruses adapted to other hosts. There is no prove of those old viruses. Bacteria though could have been proven by so-called stromatolites. These have been found in Australia and in Greenland and are about 3.6 to 3.8 billion years old. [2]

Viral infections have always been part of human kind. Viruses do not aim to kill its host limiting its own chances of survival. To increase the chances of widespread infection and viability, viruses developed the following characteristics:

1. High infectiousness.
2. Resilience against physical and environmental influence.
3. Adabtablility to new immunological circumstances via mutations.

The human (and animal) immune system is capable of fighting off viral (bacterial and fungal) infections and is able to 'memorize' special features of these pathogens. This is helpful, especially for viruses with low mutation rates. Some Viruses are prone to change their structure and genetic facilities (genetic

shift or genetic drift). These changes occur at utter random (stochastically) and follow no teleological intention. [3]

These random changes though can be triggered when medical therapeutics or preventive vaccines are administered, which lack 100% efficiancy and can not avert further infection, causing evolutionary pressure on the virus, which in turn forsters new viral variants. This renders specifically true for those pathogens prone to a certain mutation tolerance and mutate quickly.

Due to the relatively high error ratio of RNA-polymerases, RNA viruses are more prone to mutations. [4] A crucial factor is not only the pathogen's rate of mutation, but also its evolutionary ability to form antigen-varieties capable of immuneescape and unrestricted competence to replicate. Pathogens, like influenza and measels, share comparable rates of mutation. The latter however lacks antigen-varieties resluting in a likely lifelong immunity after infection or inoculation. The former mutates at a higher rate and therefore fosters an increased inclination for antigenetic drift, because most neutralizing antibodies tackle the viral hemagglutinin-protein's epitope, which is very prone of mutating. [5]

Former studies have pointed out that vaccines incapable of inhibiting transmission are likely to cause specific conditions for potent viral strains to emerge, which are in turn most harmful to invaccinated hosts inflicting even more serious illnesses. [6, 7]

Therefore, developing vaccines should not be the universal and sole sollution combatting a viral pandemic. In addition, medicinal therapeuthics and the development thereof should not be the only and acceptable way of dealing with an akute infliction, because a medicinal treatment serves merely as a repairment than a prevention. In light of possible pandamics in the future, potent prophylaxis apt to protecting against new pathogens and most importantly inhibiting resistances would be of great value. Natural occuring substances are compatible with many viral surface structures, so resistance developments can be ruled out for now.

### *Cistus x Incanus L. Pandalis*

The hoary rockrose *Cistus x incanus* L. Pandalis belongs to the genus of rock roses, which are widespread along the Mediterranean coast of Europe. There, its diverse shrub communities grow in maquis and rocky heaths on magnesium-rich, dry and rocky soils at wind-exposed sites. It grows at altitudes up to about 1000-1300 m above sea level. The evergreen, richly branched shrub reaches a growth of 0.5-1 meters in height. Its ovate-lanceolate, finely hairy leaves appear grayish-green due to numerous embedded oil droplets. The stems, pedicels and sepals are covered with white hairs. The flowers of the aromatic fragrant shrub are a bright pinkish-red and 5-6 cm wide. Due to the evolutionary process, the species and subspecies of cistus have adapted to their respective habitats in the process of adaptive radiation, which is reflected in their morphological and biochemical properties. Because of its great diversity of species and shapes, Cistus is called the 'world champion of polymorphism'. [8] The genetically similar plants of a species are shaped by their environmental conditions, resulting in different varieties. Thus, similar-looking and closely related cistus plants can differ significantly in their biochemical properties depending on location and enviromental influences. Therefore, plants of the species *Cistus x incanus* L. harvested in Turkey are by no means identical with *Cistus x incanus* L. Pandalis from Greece. Numerous scientific studies have pointed out a variety of health qualities for precisely this variety, including particularly strong antiviral effects. This can be attributed to an apparently particularly advantageous vital substance composition strongly related to the local environmental conditions. Therefore, other Cistus plants and extracts do not show the same effects.

## 2. Literature Review

Cystus Pandalis® extract is a plant-based product based on the cistus variety *Cistus x incanus* L. Pandalis, which is home to and is only been found in a limited area of northern Greece. *Cistus x incanus* L. has been part of the traditional folk medicine since the 4th Century b. c. due to its antiinflammatory, antiulcerogenic, antimicrobial and wound-healing properties. [9] What makes the Cystus extract special, is its high content of high polymer polyphenols and the specific polyphenol pattern, while monomer polyphenols account for less than 2%. [10]

Among other aspects, flavonoids and especially flavonols (myricetin, kaempferol, quercetin derivatives), condensed tannins (proanthocyanidins), hydrolyzable ellagitannins and phenolic acids (ellagic acid, gallic acid, hydroxyferulic acid) were detected (Table 1). Varieties of Cistus show significant differences in their polyphenol contents and compositions. [11]

***Table 1.*** *Polyphenols of Cistus x incanus L. Pandalis® in aqueous extracts (boiled tea 95°C; 5 min) [11].*

| Proven Substrat | Determined Concentrations (mg/100 g Tee herb) |
|---|---|
| Phenol accids | |
| Gallic acid | approx. 20 - 30 |
| Methyl gallic acid | approx. 10 - 30 |
| Hydroxyferulic acid-5-O-rhamnoside | approx. 20 - 40 |
| hydroxyferulic acid-4-O-rhamnoside | approx. 40 - 80 |
| Hydroxyferuloyl-rhamnose | approx. 40 - 80 |
| Hexahydroxydiphenyl-glucose | approx. 20 - 140 |
| Ellagic acid | approx. 05 - 20 |
| Ellagic acid-O-xylaside | approx. 05 - 20 |
| Flavanols (catechins) | |
| Gallocatechin | approx. 25 - 30 |
| Catechin | approx. 15 - 20 |
| Gallocatechin-(4α→8)-catechin | approx. 40 - 60 |
| Myricetin glycosides | |
| Myricetin-3-O- galactoside | approx. 60 - 80 |
| Myricetin-3-O-glucoside | approx. 05 - 10 |
| Myricetin-O-xyloside | approx. 05 - 10 |
| Myricetin | approx. 150 - 180 |
| Quercetin glycosides | |
| quercetin-3-O-galactoside | approx. 20 - 30 |
| quercetin-3-O-glucoside | approx. 05 - 10 |
| quercetin-3-O-xyloside | approx. 10 - 15 |
| Quercetin | approx. 35 - 40 |
| Tiliroside isomers | |
| *trans*- Tiliroside | approx. 20 - 25 |
| *cis*- Tiliroside | approx. 05 - 10 |
| Ellagitannins | |
| Hexahydroxydiphenoylglucose 1 | approx. 10 - 15 |
| Hexahydroxydiphenoylglucose 2 | approx. 40 - 50 |

Mechanism of Action of Cistus x Incanus L. Pandalis.

Many polyphenonols have been examined on their properties and antiviral effects.

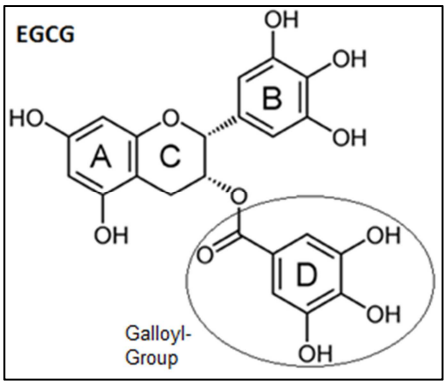


***Figure 1.*** *Epigallocatechingallat (EGCG).*

In this regard, Green tea catechins (GTC) epigallocatechin gallate (EGCG) (Figure 1) and Theaflavin (TF) have been studied extensively on their effect on various viruses like:

***Table 2.*** *Proven beneficial effects of green tea cathechins on selected viral strains [12].*

| |
|---|
| Herpes Simplex Virus (HSV) |
| Adenoviruses |
| HIV |
| Hepatitis C virus (HCV) |
| Influenza viruses |
| Zika virus |
| Chikungunya virus |
| Rotaviruses |
| Enteroviruses |
| PRRS virus |

GTC function in all mentioned viruses above as an entry-inhibitor. [12] EGCG engage broadly with surface-proteins showing effectiveness partly on viral and partly cellular structures. [12, 13] When confronted with HIV, EGCG effectively inhibits the virus from entering through binding to CD4, leaving the virus with no chance to enter the cell. As studies on the Cystus extract pointed out, polyphenols engage with viruses, keeping cells intact and cellular recepters unaffected.

Exeminations on the Cystus extract's antiviral effectiveness against HIV via the EASY-HIT-System showed an inhibition of the first step of the test. That is the reason why Time-of-Addition (TOA)-Tests were being performed as well, measuring the effect the timing of addition of the antiviral agent has on its ability to inhibit viral infection. The HIV-1 fusion inhibitor T20 and the reverse transcription inhibitor Efavirenz (Sustiva®, Stocrin®) served as reference compounds. The Cystus extract loses its inhibitory properties even before the T20 fusion inhibitor, demonstrating that the extract inhibits very early or within the first step in the HIV-1 replication cycle. [13] Subsequently, the extract's impact on the attachment of viral particles to host cells was investigated via GFP-labeled HIV-1 particles (HIV-1 NL4-3 Gag-iGFP32, GFP = green fluorescent protein). In the absence of the Cystus extract, several green flourescend cell-associated points appeared, affirming the attachement of virusparticles to cell hosts, as it was expected. In contrast, the Cystus extract treatment reduced the amount of GFP Signales per cell significantly, indicating the inhibition of virusparticles binding to the cell host by the extract (Figure 2).

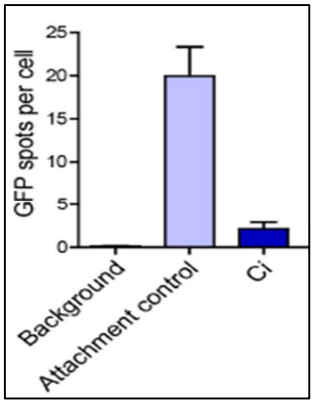


***Figure 2.*** *Cystus Pandalis® extract (Ci) blocks the cellinvasion by HIV. [14].*

In conclusion, the results show that the Cystus extract blocks the virusparticles from attaching onto the cells, therefore averting the virus from entering the cell host.

In order to ascertain weather Cystus extract's antivial components prefer attacking viruses or cell hosts, either cells or virusparticles were pretreated with the extract before infection. Preincubating the LC5-RIC-Cells with the extract showed not effect on the viral infection. On the contrary, preincubating the viral particles decreases the infection considerably and leads to even lover rates of infection compared to those rates, when adding the extract and virus simultaniously to the cells (Figure 3). These results indicate the direkt influence of Cystus extract on the viral particle. [14]

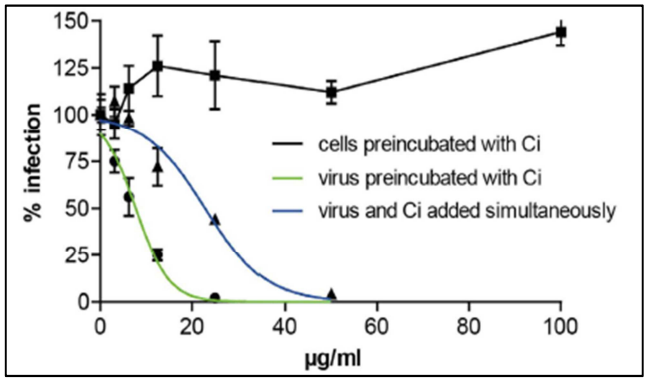


***Figure 3.*** *HIV-inhibting effect of Cystus Pandalis® Extract (Ci) aming at viral coating protein selectively. Antiviral components of Cistus Incanus extract interact solery with virusparticles, but not with the target cells. The diagram depicts the effects of the virusuinoculum (HIV-1LAI) or the target cells (LC5-RIC) on the anti-HIV-1 activity, having been preincubated with the Ci-Extract. As a controlmechanism, antiviral activity was examined under standard conditions, in which the viral inoculum and Ci extract were added simultaneously to the cells. Symbols denote the mean values for each extract dilution analyzed in triplicate. Error bars the standard deviation of the mean. Values for infection of cultures treated with Ci extract are expressed relative to untreated cultures (=100% infection). [14]*

It was analysed whether the treatment of virusparticles with

the extract has an impact on the binding of HIV-1 particles to heparin, in order to find out if the extract aims for the HIV-1 envelope glycoproteins. The viral surface protein gp120 arranges the binding of HIV-1 to heparin. Those viruses pretreated with the Cystus extract, no binding to heparin was found in the assay. These results suggest that the Cystus extract blocks the gp120 mediated binding to heparin. [14] Similar to EGCG, the viral structure gp120 is being cloged by the extract preventing the virus from entering the cell. [14]

EGCG and theaflavin digallates (TF3) (Figures 4A and B) were shown to bind to the hemagglutinin of influenza viruses, preventing cell entry. [15] The same effect was observed, when Cystus extract was studied. [10]

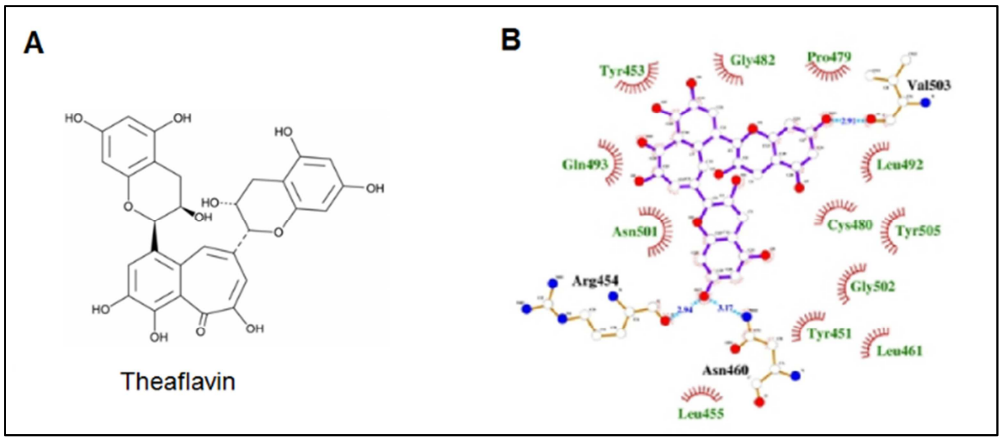


***Figure 4.*** *A: Structural formula of theaflavin, B: Theaflavin' interaction with the receptor-binding domain of the S protein of SARS-CoV-2.*

Recent studies have shown that EGCG and theaflavin (TF1) bind to the S-protein of SARS-CoV-2 averting the S-protein interacting with the ACE receptor. [16] Due to resembling polyphenols comprised in the Cystus extract, this mechanism could be plausible in this case. [9, 11]

The antiviral properties of catechins correlate with the number and position of hydroxyl groups on the B ring and the presence of a galloyl group. [12] A large number of the polyphenols of Cystus extract meet these requirements, so the antiviral effect is reasonable. [11]

The descriped machanism entails no development of resistances, which is a remarkable advantage. [10, 14] This understanding pointd out a study dealing with highly mutating HI-Viruses. In the cell model, no resistant viruses emerged even after 48 rounds of infection over 24 weeks during which the virus was exposed to the Cystus extract. Even after the end of all passages, the extract kept being fully effective against HIV-1 (Figure 5).

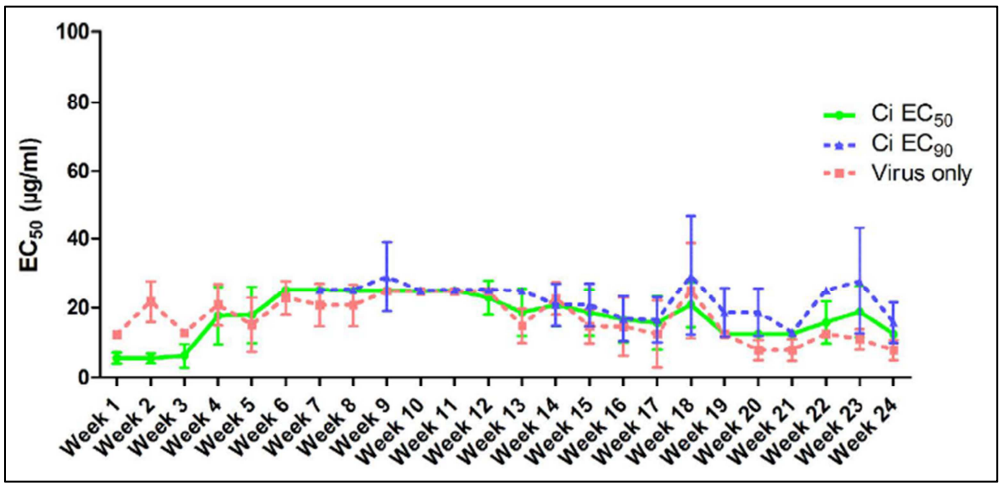


***Figure 5.*** *No HIV-1 resistance detected when Cystus Pandalis® extract (Ci) was present in 24 weeks. Virus was passaged with or without Ci extract for 48 runs (i.e. 24 weeks) and monitored each week for sensitivity of inhibition by Ci extract. For each passage with Ci extract, LC5-RIC target cells were exposed to HIV-1LAI virus in the presence of Ci extract for one day, followed by expansion of escape virus for 2-3 days without Ci extract. Long-term viral passage was performed with two concentrations of Ci extract corresponding to the EC50 and EC90 inhibitory concentrations relative to the original viral inoculum, respectively. Passage in the presence of the higher Ci extract concentration (Ci EC90) was initiated with viruses collected after 6 weeks of passage with the lower Ci extract concentration. Simultaniously, the virus was passaged as a control without Ci extract (line graph virus only). The Virus collected at every other passage was tested for inhibition by Ci extract in infection assays with different concentrations of Ci extract (100 µg/ml) assayed up to 3.125 µg/ml; twofold serial dilutions) or without extract (= 100% infection). The symbols represent the mean EC50 determined for each virus sample in three independent experiments, and the error bars represent the standard deviation from the mean. [10]*

These examinations approve prior experiments on influenza conducted in 2007 showing no development of resistances, despite repeated passaging. As early as that time it was summarized that Cystus extract does not cause resistance development. [10]

This study has the aim to merge the evidences and the results of prior investigations to build a reliable frame of the Cistus × Incanus L. Pandalis to induce further examinations.

# 3. Antiviral Characteristics of Cistus x Incanus L. Pandalis

## *3.1. In-vitro Studies*

Derived from *Cistus x incanus* L. Pandalis, the extract Cystus Pandalis® has been subject to several examinations always pointing out its positive results concerning its antiviral potential. In-vitro examinations included all recently studied pathogens and was never restricted to any viral families or subtypes.

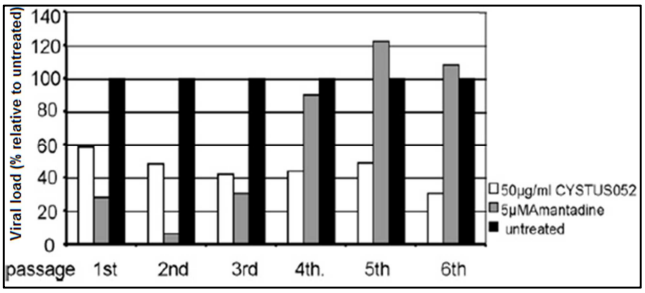


***Figure 6.*** *Cystus Pandalis® extract shows no tendency of inducing a virus resistance. MDCK cells were infected with Influenza A virus strain FPV (MOI = 0.01) and either left untreated or treated with the indicated amounts of Cystus Pandalis® extract or Amantadine. Supernatants were collected 24 hours after infection and used for anather infection in the second round of the study. After infection, cells were left untreated or treated again with the indicated amounts of Cystus Pandalis® extract or amantadine. This procedure was repeated six times. Supernatants were assayed for offspring viruses yield in standard plaque titrations. The viral yield of MDCK-treated cells was set as 100% (y-axis). [10].*

***Table 3.*** *Synopsis of in vitro studies on the antiviral activity of Cistus x incanus L. Pandalis.*

| Virus | Cellsystem / Cytotoxicity | Antivirale Potency | Source |
|---|---|---|---|
| Influenza virus A (H1N1, H5N1, H7N7) | MDCK, A549 No cytotoxic effects at 50 µg/ml. | EC50 H7N7 = 10 µg/ml | [10] |
| Rhinoviruses (HRV14) | | EC50 no further specified | [10] |
| Influenza virus A (H1N1, H5N1, H7N7). Isolates: A/Hamburg/4/ 2009 A/mute swan/Germany/ R1349/07 A/mallard/Bavaria/1/2006 A/common buzzard/Bavaria/ 11/2006 A/common buzzard/Bavaria/ 2/2006 A/great crested grebe/ Bavaria/22/2006 A/goldeneye duck/Bavaria/ 19/2006 A/goosander/Bavaria/20/2006 | MDCK | EC50 H1N1 = 3.58 µg/ml H5N1 = 1.53 – 18.88 µg/ml For specific influenza A isolates BB1 and BB2, the EC50 averaged 72.22 µg/ml. | [17] |
| HI-Viruses (HIV-1, HIV-2) | LC5-RIC, PBMC Cellviability (PMBC): CC50 > 250 µg/ml | EC50 (LC5-RIC): HIV-1 = 6.04 – 15.06 µg/ml HIV-2 = 14.05 µg/ml EC50 (PBMC): HIV-1 = 4.9 – 20.01 µg/ml | [14] |
| Ebola virus | | EC50 = 5.2 µg/ml | [14] |
| Marburg virus | | EC50 = 4.7 µg/ml | [14] |
| *MERS-Corona* | *A549. Cellviability at 62.5 µg/m: A549 > 50% Huh7 > 90%* toxic only above 250µg/ml | *EC50 = 60µg/ml* | *unpublished* |
| SARS-CoV-2 (Wild type) | Vero E6, toxic only above 250µg/ml | EC50 = 1.94µg/ml | [18] |
| SARS-CoV-2 (Alpha) | Caco2 CC50 > 400 µg/ml | EC50 = 48.9 µg/ml | [19] |
| SARS-CoV-2 (Beta) | | EC50 = 45.2 µg/ml | [19] |
| SARS-CoV-2 (Delta) | | EC50 = 101 µg/ml | [25] |

### *3.1.1. Influenza Viruses*

Deeper insights into the antiviral properties of Cystus Pandalis® extract has found Ehrhardt et al. and Droebner et al. [10, 17]. They could demonstrate that this extract was effective against H5N1, H7N1, even against the H1N1 virus and other types of influenza A. A significant reduction of virus titres was shown in all cases. In contrast to the comparable administered virustatic Amantadine, no cases of resistances on influenza viruses were found, which was remarkable (Figure 6).

The Cistus Pandalis® extract was effective even against those H5N1 virus strains that were resistant to oseltamivir (Tamiflu®). At the effective dose of 50 µg/ml, the extract showed no adverse effects on cell viability, metabolism, or proliferation. The using of these plant extracts in traditional medicine for thousands of years without any reported complications supports this fact. [17] At the molecular level, the protective effect of the extract appears to be mainly due to the binding of the polymeric polyphenol components of the extract to the virus surface. [10, 18]

### *3.1.2. HI-Viruses*

Cystus Pandalis® extract inhibited clinical HIV-1 and HIV-2 isolates. It is remarkable to note that the extract is able to avert viral isolates containing multiple resistances, confirming its broad antiviral effect against HIV. The antiviral impact was highly selective against virusparticles hinderung the primary attachement of the virus on the cellular surface. This extract demonstrated prevented viral envelope proteins from binding to heparin. Bioassay-guided fractionation showed that the extract contained numerous antiviral compounds and was therefore not prone to induce viral resistance. In fact, no resistant viruses occurred during the 24-week continuous propagation of the virus in the presence of the Cystus extract at all. [14]

### *3.1.3. Other Viruses*

The studies mentioned above have shown that Cystus Pandalis® extract also prevented infection by viral particles pseudotyped with Ebola and Marburg virus envelope proteins. According to the authors, this indicates the antiviral activity of the extract encompassing emerging viral pathogens, too. [14]

### *3.1.4. SARS-CoV-2 (Wild Type, Alpha, Beta, Delta)*

Cystus Pandalis® extract proved to be effective against the wild type as well as the variants alpha, beta and delta. For every variant except for the wild type, the same test setup was used (see below). The IC50 was 48.9 µg/ml (alpha), 45.2 µg/ml (beta), and 101 µg/ml (delta), respectively. In addition, the MTT assay showed Cystus extract having no negative effect on cell viability at the effective test concentrations and well beyond (Figures 7 and 8). [18, 19, 25]

Examining the wild type, even an EC50 of 1.94 µg/ml was determined. However, a different experimental set-up was chosen there. It was a focus forming assay using Vero E6 cells. The reduction of the infection rate was measured by counting the number of focus forming units in relation to the extract concentration (Figure 9). [18]

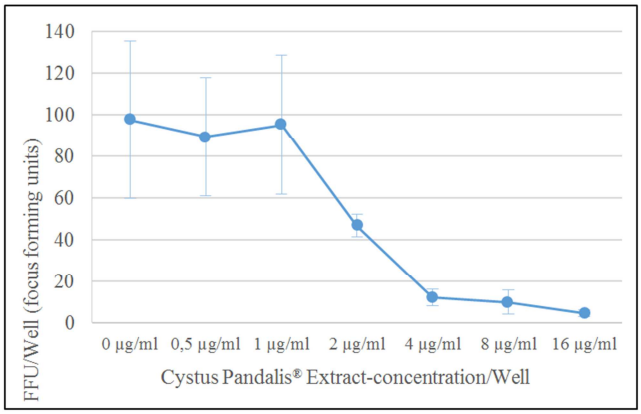


***Figure 7.*** *Concentration-dependent antiviral effects of Cystus Pandalis® extract on SARS-CoV-2-induced cytopathic effect (CPE) determined 48 hours after infection in Caco2 cells infected with the British (B.1.1.7) and South African (B.1.351) SARS-CoV-2 variants at a multiplicity of infection (MOI) of 0.01. Cystus Pandalis® extract prevented cell infection in both variants and showed no negative effects on cell viability in the MTT assay (green). [19].*

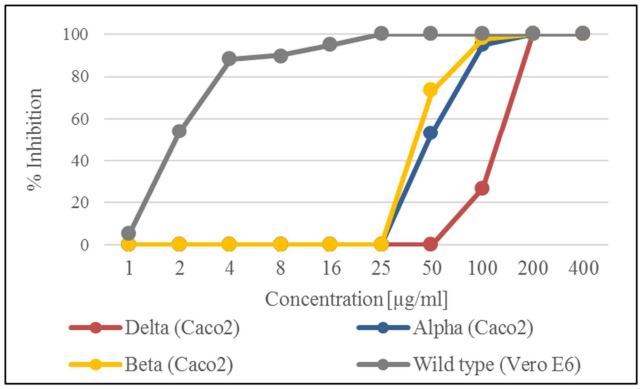


***Figure 8.*** *Concentration-dependent antiviral effects of Cystus Pandalis® extract on SARS-CoV-2-induced cytopathic effect (CPE) determined 48 hours after infection in Caco2 cells infected with the British (B.1.1.7), South African (B.1.351), and Indian (B.1.617.2) SARS-CoV-2 variants at a multiplicity of infection (MOI) of 0.01. Cystus Pandalis® extract prevented cell infection in all three variants. [25].*

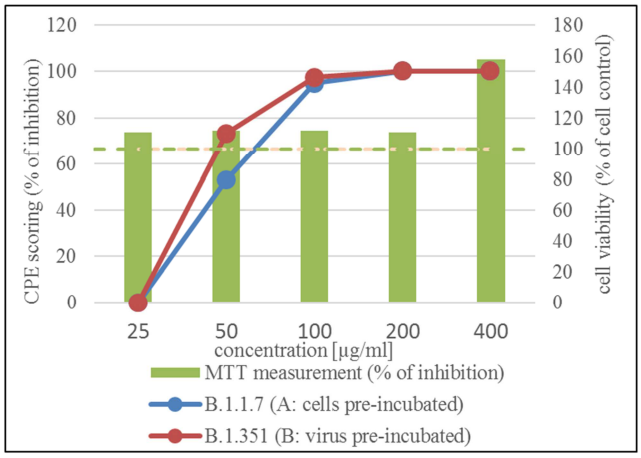


***Figure 9.*** *Reduction of SARS-CoV-2 infection rate in relation to CPE concentration. Number of FFU/well detected in relation to the tested concentration of Cystus Pandalis® extract after infection of Vero E6 cells with SARS-CoV-2 (mean and standard deviation; n=2). [18].*

The binding of SARS-CoV-2 to human cells appears to occur via the ACE receptor ("angiotensin-converting enzyme 2") through the cell wall. [26] To determine the manner in which Cystus extract exerts antiviral activity against SARS-CoV-2, a binding assay was performed. The extent to which the Cystus extract can interfere with the binding between the spike protein or receptor binding domain (RBD) and ACE2 was investigated.

Firstly, the ACE2 enzyme was placed together with the solubilized Cystus extract and SARS-CoV-2 spike S1 protein in the sample tube. This solution was subsequently incubated. Finally, the sample was loaded with a chemiluminescent substance, which can be measured by a chemiluminescence reader. Spike S1 neutralizing antibody (from Bioscience company) was used as a positive control.

For the comparative experiment, the same setup was chosen, but here this approach was treated with anti-mouse Fc-HRP - a specific antibody of the receptor-binding domain (RBD). Again, the sample was loaded with a chemiluminescent substance that can be measured by a chemiluminescence

reader. Also, Spike S1 neutralizing antibody (from Bioscience company) was used as a positive control. Cystus extract reduced the binding between ACE2, spike protein and RBD in a concentration-dependent manner. At 250 µg/ml, the extract reduced ACE2 spike binding and ACE2 spike-RBD binding by approximately 60% each (Figure 10). There were no cytotoxic effects of the extract at the above mentioned test concentrations. [25] Thus, it can be proved that the application of Cystus extract decreases the attachment of SARS-Cov-2 to the receptorbinding site on the ACE2 enzyme in a dose-dependent manner. The extract administered during the trail did not induce cytotoxic effects.

### *3.2. In-vivo Studies*

The antiviral activity of *Cistus x incanus* L. Pandalis and the Cystus Pandalis® extract derived from it were investigated in several in-vivo studies. This includes mouse model studies as well as larger clinical studies on humans. (Table 4).

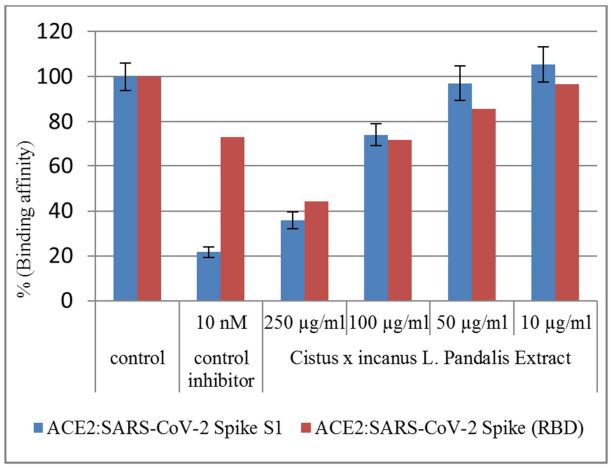


***Figure 10.*** *Effect on the interaction between ACE2 and SARS-CoV-2 Spike S1 and between ACE2 and SARS-CoV-2 Spike RDB. The data represent the mean of n=8 ± SD (the control binding has been set to 100%).*

***Table 4.*** *Synopsis of in vivo studies on the antiviral activity of Cistus x incanus L. Pandalis.*

| Year | Summary of the Study | Design | Source |
|---|---|---|---|
| 2007 | Cystus Pandalis® shows anti-influenza virus activity in mice. Mice treated with Cystus Pandalis® did not develop disease. In four experiments, 18 out of 20 mice in the Cystus Pandalis® group survived, while 7 out of 15 mice in the control group died. | In-vivo animal model (n=20) | [20] |
| 2009 | The score of subjective symptoms decreased significantly during the course of treatment with Cystus Pandalis®, while treatment with placebos resulted in a less significant decrease of symptoms. | In-vivo prospective, randomized, placebo-controlled clinical Study (n=160) | [21] |
| 2009 | The score of subjective symptoms decreased during the course of treatment with Cystus Pandalis®, while treatment with green tea resulted in a less significant decrease of symptoms. | In-vivo, clinical Study (n=300) | [22] |
| 2019 | Cystus Pandalis® proved to be reliable for the treatment and prevention of respiratory diseases in terms of efficacy and safety. | Retrospek-tive, practi-cal applica- ting, sur-veilled by a physician. (n = 500) | [23] |
| 2020 | Prophylactic use of Cystus Pandalis® (daily 3x2) for at least 6 weeks. At the end of the study, none of the participants has got in-fected with SARS-CoV-2. | Retrolective Study surveilled by a physiscian for 6 weeks (n = 125) | [24] |

Shortly after the first positive in-vitro studies regarding influenza viruses, studies on animal models were initiated. A mouse infection model confirmed the antiviral activity against a highly pathogenic Avian Influenza A Virus (H7N7). The in-vivo treatment was performed with an aerosol formulation, as the bioavailability of high molecular weight polyphenols is low. For this purpose, a novel monitoring system was used to monitor the mice infected with Influenza A Viruses by recording their body temperature and gross motor activity. The mice treated with Cystus extract did not develop any disease, did not show differences in their body temperature or gross motor activity and did not show histological changes in the epithelial cells of the bronchiolus. In four experiments, 18 out of 20 mice in the Cystus extract group survived, while 7 out of 15 mice in the control group died. [20]

In a placebo-controlled clinical study on humans with a total of 160 participants, Cystus extract was also studied under practical conditions. Participants suffering from an acute respiratory infection (58% were virally ill) received Cystus extract daily in the form of 6 x 2 lozenges. Both the subjective symptoms of the disease and the CRP value (inflammation value) decreased significantly faster in the verum group, indicating a shortened duration of infection. On average, the Cystus Pandalis® extract reduced the duration of illness by two days. [21]

Subsequently, another paper investigated the clinical effect of Cystus extract compared to green tea in 300 patients with upper respiratory tract infections. In this study, a total of 300 patients (277 completers) were observed who were treated with Cystus Pandalis® extract in lozenges compared to treatment with an extract of green tea. Using a predefined scale, the patients were asked to rate their subjective severity of their target symptoms. The subjective symptom score decreased over the course of treatment with Cystus extract, while treatment with green tea showed a less significant decrease in symptoms. [22]

A total of 500 responses from physicians were evaluated as part of a survey on the usage of the traditional herbal medicine Cystus Pandalis® lozenges. 61% reported the drug was used for the treatment of acute respiratory tract infections and 19% for the prophylaxis of acute respiratory tract infections. The indications flu/influenza prophylaxis, flu/influenza therapy and irritable cough/hoarseness were mentioned in roughly equal proportions, in addition to those mentioned above. 86% reported positive feedback on tolerability and regarding effectiveness, 278 of 285 physicians (97.5%) indicated a well

received Cystus Pandalis® efficacy. [23]

In a recent retrolective study conducted to examine the relation between administering Cystus extract and the incidence of COVID 19, 125 volunteers used Cystus extract in lozenge form (3 x 2 daily) for at least 6 weeks. No participant was affliced with SARS-CoV-2 during the whole study. It is most remarkable that nine participants had one family member living in the same household, who contracted COVID-19 (positive PCR test, not belonging to the study in progress). These nine participants had started prophylaxis approximately seven to ten days prior to exposure to the ill family members. None of these subjects got an infection with SARS-CoV-2, which was confirmed by PCR testing. [24]

# 4. Discussion

As part of emperical medicine, Cistus has been used for over 2000 years. Since it has been utilized mainly since the modern times as a remedy against infections, it was obvious to look at its effects on bacterial and viral growth. This paper summarizes the results of previous research on the influence of Cystus Pandalis® extract on virus replication.

Over the past fifteen years, studies of Cystus extract have been undertaken investigating its effects against influenza viruses, rhinoviruses, HIV viruses (HIV-1, HIV-2), Ebola virus, Marburg virus, MERS-Corona, and SARS-CoV-2 (wild type, mutants alpha, beta, and delta). These studies have shed a light on the extracts potent efficiancy against all investigated virus species [10, 14, 17-25]. Most of the tests were carried out in-vitro, but some studies were also performed in-vivo, performed on either patients or laboratory animals [20-24]. Infection of cell cultures by the applied viruses was completely prevented in-vitro without any the extract causing any damage to the cell cultures. These in-vivo studies showed that the application of Cystus extract largely prevented infection of the test subjects, while comparable subjects in a similar life situation showed infections more frequently. This fact confirms and underlines centuries of experience with plant extracts of Cistus incanus in forms of tea, extracts, ointments and wound dressings, and its utilitization. It has continuously been described as having positive effects with largely no side effects. Furthermore, the studies have shown no development of resistance to date on the part of the viruses investigated [10, 14].

The in-vitro studies took place on different cell cultures (MDCK, A549, LC5-RIC, PBMC, Vero E6, Caco2). Similar positive results were found in all series of experiments, that is why we assume a broad effect with different viruses and with different cell types. Some in-vivo studies could not take place prospectively in a controlled setting as planned due to a lack of permission by the ethics committees and were therefore only possible as retrolective studies or as observational studies. Of course, a BIAS is to be expected here. With clear results, one can at least assume a helpful application despite this BIAS.

The substance groups responsible for the effect are probably polyphenols, which are present in high concentration in this extract. The particularly high proportion of polymeric polyphenols (over 90%) and the relatively low proportion of monomeric polyphenols (about 2%) seem to be the exceptional characteristic of this extract. Other substances rich in polyphenols have not been able to demonstrate these protective effects, as studies on green tea extract and other teas such as black tea have pointed out. The insufficient effect of these substances may be partly due to the fact that in these extracts the proportion of polymeric polyphenols is lower, while the proportion of monomeric polyphenols is higher than in the Cystus extract.

Drugs used in conventional medicine against influenza infections (e.g. amantadine = PK-Merz®, various generics) and also neuramidase inhibitors (Tamiflu®, Relenza®) showed temporary effects, but also rapidly increasing resistance of the viruses to these drugs. Therefore, these drugs are not recommended for therapy, nor are they suitable for prophylaxis. In contrast, Cystus extract can largely avoid the development of resistance when this natural mixture of substances is used. This seems to be an advantage of thos extract over the use of chemically defined monosubstances. Having being administered for more than 2000 years, no resistance developments caused by the extract, or the plant respectively, have ever been reported, although it must of course be mentioned that reports on resistances to chemotherapeutic agents have only arisen in the last 60 years and therefore investigations in this respect have only been undertaken fairly recently.

The mechanisms of action cause an inhibition of entry by blocking the attachment sites. In the case of SARS-CoV-2, for example, it is assumed that the Cystus extract coats the spike proteins, preventing the viruses from binding to human cells (e.g. in the oral pharynx or respiratory tract), thus preventing infection [25]. This binding of the virus to human cells appears to occur via the ACE receptor (‘angiotensin-converting enzyme 2’) within the cell wall. [26] In order to determine the manner in which Cystus extract exerts antiviral activity against SARS-CoV-2, a binding assay was performed. The goal was to find out the extent to which Cystus extract can interfere with the binding process between the spike protein or receptor binding domain (RBD) and ACE2.

The extract reduced ACE2 spike binding and ACE2 spike RBD binding by approximately 60% respectively. There were no cytotoxic effects of the extract at the above mentioned test concentrations. [25] Therefore, it can be proved that the application of Cystus extract decreases the attachment of SARS-CoV-2 to the receptorbinding site on the ACE2 enzyme in a dose-dependent manner. The used dosage of Cystus extract did not induce cytotoxic effects.

This also demonstrated that Cystus extract affects the direct binding of RBD, preventing SARS-CoV-2 from binding to the cellular ACE2 receptor and preventing the virus from entering the cell through this route in the first place. [25] It is unclear though whether the ingredients of Cystus extract also target other structures of SARS-CoV-2, such as E protein (envelope, membrane protein), M protein (membrane protein), or N protein (nucleocapsid protein).

Buehring favored a prophylactic local application against potential viral infections already in 2014. [27] Studies on coronaviruses, their infection behavior and human-pathogenic effects were only rudimentary at that time. A SARS-CoV-2 pandemic could not have been forseen. It was not yet possible to assess the possibility of using Cystus extract for SARS-CoV-2 infections on humans. Since the viruses (influenza, SARS-CoV-2) are mostly absorbed through the mucous membranes of the upper respiratory tract, a local application in this area (mouthwash or gargle, lozenges) seems to be a plausible, useful, local practice.

The application is simple and safe: Cystus Pandalis® extract is available as a lozenge or in the form of a decoction as a mouthwash. Prophylactic use in times of infection and viral epidemics/pandemics at any stage is easily feasible. People tolerate this extract very well. It has shown no side effects, yet. It is freely available. So far, there are no known contraindications or interactions with other medications. People of any age and sex can use this extract.

At a time, when SARS-CoV-2 infections have not yet subsided, when there are deficiencies in vaccination campaigns, when there are many citizens who do not want to be vaccinated with the approved vaccines or who are not able to be vaccinated due to health reasons, one have to search for alternative options.

It gives hope having a viable and likely option for prophylaxis at our disposal is not only sensible, but in case of possible waves of infections in the future, worthwhile.

## 5. Summary

At Cystus Pandalis® has been utilized in empirical medicine for a long time. For fifteen years, studies have been conducted on the effects of Cystus Pandalis® extract against various types of viruses. Against all types of viruses investigated, the extract's efficiancy was significant. Most tests were carried out in-vitro, some studies were also carried out in-vivo on patients and laboratory animals. In physiological doses of Cystus extract, infection of cell cultures by the applied viruses was completely prevented in-vitro, without the cell cultures being damaged by the Cystus extract. In the in-vivo studies, the application of Cystus extract largely prevented infection of the subjects, while subjects living in similar life situations showed infections more frequently. These studies showed that the viruses have not yet developed any resistance.

The substance groups responsible for the effect are probably polyphenols, which are present in high concentration in this extract. The particularly high proportion of polymeric polyphenols (over 90%) and the relatively low proportion of monomeric polyphenols (about 2%) seem to be the exceptional caracteristic of this extract. So it blocks the cellular attachment sites and inhibits the virus from entering. As it is for SARS-CoV-2, it seems plausible that the extract encases the Spike-Protein to prevent the virus from attaching to the human cell (e.g. in the mouth, throat or respiratory tract) and therefore avert infection.

The Cystus Pandalis® extract is available as a lozenge or as a mouthwash. A prophylactic application in times of infection and viral epidemics/pandemics in any phase is easily viable. The extract is well tolerated, has shown no side effects, yet, and is freely available. So far, there are no known contraindications or interactions with other medications. The extract can be used by people of any age and sex.

In times, when SARS-CoV-2 infections are still ongoing and there are many people who do not want or are not allowed to be vaccinated, it is viable to make use of a substance that is helpful in infection prophylaxis. This should also be proposed in view of future infection situations and possibly emerging pandemics.

## Note

In this publication, the term Cystus extract always refers to Cystus Pandalis® extract.

## Limitations

To date, no randomized, controlled, double-blind studies have been conducted in human subjects. Comparable study protocols failed in each case and were rejected by the ethics committees. Thus, studies have largely been open-label or retrolective. Approval of a study meeting these criteria by a German ethics committee is not to be expected for the time being.

## Conflict of Interest

The author hasn't any possible conflict of interest.

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