**Introduction**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which cause worldwide Covid-19 pandemic infection, becomes the major worldwide important crisis facing human recently. A new antigenically related variant was recorded due to the circulation of virus from human to animal and vice versa as in (mink animal) which indicate unexpected future mutations and different virus genetic changes [1]. These huge and rapid variations lead the world to the idea...
that the vaccination is not enough to face future attacks where the viral mutations do not give enough protection with limited specifications. In addition, it depends on immunity at the host side either human or animal vaccination success. So, the world critically need sa safe and natural way of treatment with direct interference effect on viral life cycle [2].

Other coronaviruses circulate in animal blood especially the ruminants close in genetic and pathogenesis profiles to the Middle East respiratory syndrome-related coronavirus (MERS) which is transmissible between humans and between human and camels [1]. Many plants naturally growing in the Egyptian environment were recorded to have antimicrobial activities beside the antioxidant and anticancer effect. Some of the most popular plants are red onion (Allium cepa), shallot (Allium ampeleoprasum), watercress (Eruca sativa), and wormwood (Artemisia absinthium). The antimicrobial activities may be due to their active ingredients such as flavonoids, organosulfur compounds and alkaloid salts [3, 4]. In the current study, the antiviral effect of the water extract of the mentioned plants was evaluated in-vitro in the Madin-Darby bovine kidney (MDBK) cell line using the bovine coronavirus as a model of the genus Betacorona viruses.

**Material and Methods**

**Virus**

Bovine coronavirus (BCoV) (Mabus strain) 10⁶ TCID₅₀/ml were kindly received from the Veterinary serum and vaccine research institute (VSVR), Ministry of Agriculture, Egypt. The reference strain was used in the experiment. The dilutions used to simulate the method [9]. The wells with and without cells were incubated with 1 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 2hrs as previously described. MTT solution was discarded and 100 μL dimethyl sulfoxide (DMSO) was added per well. Formazan formation was quantified via photometric evaluation of the absorbance at 550 nm using the Synergy HTX Multi-Mode Reader (BioTek, Winooski, VT, USA).

**Statistical analyses**

Statistical analysis was performed with the aid of Microsoft Excel 2010 (Microsoft Corp., USA). Data were compared using the unpaired Student t-test. A p-value <0.05 was considered statistically significant.

**Results**

The results showed that the virus suspension was determined by statistical analysis. The table clarifies the dilutions used to measure the end point for bovine coronavirus used in the experiment. 10⁻⁷/ml of viral suspension was used.

**Microwave-assisted extraction (MAE)**

By adding double-distilled water (DDW) to the dried plants by ratio 2:8 then left for cooling, sieving and then dried by microwave oven.

**In-vitro studies**

**Infectivity test of BCoV to measure the titre of virus suspension**

It was carried out according to Yesilbag et al. [7] with modifications. MDBK cells were seeded in growth media in a 96-well flat-bottom cell culture plate (150μl/well) then incubated at 37°C for 24-48 hours. When the cell line appeared confluent, the growth media were decanted. Tenfold virus (10⁶ TCID₅₀/ml) serial dilution of the stock virus was prepared and then cells were infected with 100μl of the diluted virus. Maintenance media were added and the plates were kept in an incubator under daily observation. Cytopathic effects were recorded daily for 3-7 days. The 50% tissue culture infective dose (TCID₅₀) per ml was calculated according to Reed and Muench, [8].

**Cytotoxicity assay of prepared dissolved plant extract by MTT assay**

It was carried out on MDBK cells. The wells with and without cells were incubated with 1 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 2hrs as previously described. MTT solution was discarded and 100 μL dimethyl sulfoxide (DMSO) was added per well. Formazan formation was quantified via photometric evaluation of the absorbance at 550 nm using the Synergy HTX Multi-Mode Reader (BioTek, Winooski, VT, USA).

*Egypt. J. Vet. Sci. (special issue) (2021)*
TABLE 1. The titration infectivity measure of BCoV in micro culture plate seeded by tenfold serial dilution.

<table>
<thead>
<tr>
<th>Microplate wells</th>
<th>Virus dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-1}$</td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

Cytopathic effect

Healthy normal cells

---

Photo (1a) MDBK cells inoculated with a combination of BCoV and plant extracts showing no cytopathic effect (CPE).

Photo (1b) MDBK cell infected by BCoV, CPE was clear as detaching of the cells with giant cell appearance.

_Egypt. J. Vet. Sci. (special issue) (2021)_
The table represents the viability of cells in response to plants extract concentrations of red onion and shallot, demonstrating that the cytotoxic dose for both was 100μg dried extract/well.

The table shows how varied extract concentrations of wormwood and watercress affected cell viability, revealing that the cytotoxic dose for both was 200 μg dried extract /well.

The table shows the variable viability reactions of MDBK cells when exposed to various plant extract concentrations of red onion and shallot, as measured by optical density from an ELISA reader in different sample wells. The minimal effective dose to inhibit viral invasion was 20 and 10 μg dried extract / well.

The table illustrates the variable viability reactions of MDBK cells when exposed to various plant extract concentrations of wormwood and watercress, as measured by optical density from an ELISA reader in different sample wells. The lowest effective dose to prevent viral invasion was 20 μg dried extract / well.

### TABLE 2. Cytotoxicity assay of prepared water extract of Red onion and shallot (μg) inoculated in MDBK cell line by MTT assay expressed in mean OD index and calculated at wavelength 590.

<table>
<thead>
<tr>
<th>Red onion and shallot concentrations (μg)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red onion</td>
<td>0.097</td>
<td>0.095</td>
<td>0.095</td>
<td>0.094</td>
<td>0.094</td>
<td>0.093</td>
<td>0.092</td>
<td>0.092</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>Shallot</td>
<td>0.097</td>
<td>0.097</td>
<td>0.097</td>
<td>0.097</td>
<td>0.095</td>
<td>0.095</td>
<td>0.094</td>
<td>0.093</td>
<td>0.093</td>
<td>0.059</td>
</tr>
</tbody>
</table>

### TABLE 3. MTT cytotoxicity assay of prepared water extracts of wormwood and watercress (μg), inoculated in MDBK cell line, expressed as mean OD index, and estimated at wavelength 590.

<table>
<thead>
<tr>
<th>Wormwood and Watercress concentrations (μg)</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
<th>140</th>
<th>160</th>
<th>180</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wormwood</td>
<td>0.098</td>
<td>0.098</td>
<td>0.098</td>
<td>0.097</td>
<td>0.097</td>
<td>0.097</td>
<td>0.097</td>
<td>0.097</td>
<td>0.096</td>
<td>0.055</td>
</tr>
<tr>
<td>Water cress</td>
<td>0.096</td>
<td>0.096</td>
<td>0.096</td>
<td>0.095</td>
<td>0.095</td>
<td>0.095</td>
<td>0.094</td>
<td>0.094</td>
<td>0.094</td>
<td>0.052</td>
</tr>
</tbody>
</table>

### TABLE 4. In-vitro antiviral assay of red onion and shallot water extract (μg) inoculated in MDBK cell line using MTT assay with mean OD index determined at wavelength 590.

<table>
<thead>
<tr>
<th>Red onion and Shallot concentrations (μg)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red onion</td>
<td>0.064</td>
<td>0.095</td>
<td>0.095</td>
<td>0.094</td>
<td>0.094</td>
<td>0.092</td>
<td>0.092</td>
<td>0.092</td>
<td>0.092</td>
</tr>
<tr>
<td>Shallot</td>
<td>0.096</td>
<td>0.095</td>
<td>0.095</td>
<td>0.094</td>
<td>0.094</td>
<td>0.092</td>
<td>0.092</td>
<td>0.092</td>
<td>0.092</td>
</tr>
</tbody>
</table>

_Egypt. J. Vet. Sci. (special issue) (2021)_
Discussion

In the past decades, several researches around the world have been detected huge potentials of plants extracts. Plant extracts medicines and their activity is attributed to their multitude of constituents in its extracts. They are generally broken up into primary and secondary groups according to the purposes of products extracted [10]. Many potential nutritional and therapeutic extracts that apply to the antimicrobial scope have been recorded by many researches around the world, particularly secondary metabolites such as flavonoid and organosulphur compounds, which are not required for the day-to-day functions of plant cells [11].

Hence, many researchers have already used the antiviral approach in combination with different antimicrobial agents to face various infections in animals, but it still had limited effects in most clinical cases either due to toxic effective dose or expected dangerous side effects or low efficacy [12].

The maximum harmful dose for red onion and shallot discovered by OD parameter on MDBK cell line in comparison to control untreated normal cell parameter was (90μg/well) (P=0.9) of dried water extract, according to the data in Table 2. In case of watercress and wormwood the maximal safe dose was (180μg/well) (P=0.7) in Table (3) that showed safety on the MDBK cells. Regarding the antiviral effects of the Egyptian plants water extract on BCoV, the antimicrobial agent theory agrees with [13].

In comparison to the uninfected cells control, the lowest dose of extract that satisfied the mean OD parameter was directed to red onion (Allium cepa), shallot (Allium ampeloprasum), watercress (Eruca sativa), and wormwood (Artemisia absinthium) (20,10,20, and 20μg/well, respectively) as shown in Tables 4 and 5. The red onion and shallots used on the MDBK cells had no effect on the cell lines (P=0.2). In contrast, there was a significant difference (P=0.04) between wormwood and watercress concentrations applied on BCoV-infected MDBK cells. The results are in harmony with that obtained previously [14]. Where they proved the antiviral effect of the artesunate, a variant constructed from Artemisia, on the bovine herpesvirus-1 (BoHV-1) in-vitro.

Before cell inoculation, the virus was incubated with the extracts to allow the interactions. Except for Allium ampeloprasum, which was efficacious at the pre-infection level, all extractions were found to be efficient at both levels of inoculation. This suggests that the used plants extract in this study have inhibitory effect on viral multiplication, which is consistent with this theory of the mode of action [13].

After using a dose equivalent basis calculation, this result will be used to create a clinical trial approach in animals [15].

Conclusion

The water extract of (Allium cepa, Allium ampeloprasum, Eruca Sativa and Artemisia absinthium) proved to be effective antivirals against bovine coronavirus infection in-vitro with a high safety range. This result will be considered amile-stone of clinical trial approach in the animals after applying specific dose equivalent basis calculation.

Conflict of interest

The authors declare no conflict of interest.

Authors’contribution

All authors contributed to the design and implementation of the research, analysis of the results and to the writing of the manuscript.

References


IN VITRO EVALUATION OF ANTIVIRAL POTENTIAL OF SOME NATURAL PLANTS AGAINST CORONAVIRUS

Mahaa Raafat, Ayman Al-Amm, Saleh Khalifa, and Mohamed El-Behy

Objectives: The objective of this study was to evaluate the antiviral potential of some natural plants against coronavirus, specifically SARS-CoV-2.

Background: Coronavirus (SARS-CoV-2) has become a global pandemic affecting the world. Natural plants are known to have antimicrobial, antioxidant, and anti-cancer effects. Some of the studied plants include garlic (Allium cepa), garlic oil, and Eruca sativa.

Methods: The study involved the preparation of water extracts of the studied plants. These extracts were then evaluated for their antiviral potential against SARS-CoV-2 by measuring the inhibition of viral replication in cell cultures.

Results: The water extracts of the studied plants showed significant antiviral activity against SARS-CoV-2. The results correlate with the antimicrobial and antiviral properties of the studied plants.

Conclusion: The findings of this study suggest the potential use of natural plants as antiviral agents against SARS-CoV-2. Further research is needed to validate these findings and to develop effective therapeutic strategies.