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Effect of Asphodelus Microcarpus on Low Pathogenic Coronavirus 229E

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Abstract

Human coronavirus and several picornaviruses are responsible for worldwide epidemic outbreaks, thus representing a heavy burden to their hosts. Coronaviruses (CoVs), enveloped positive-sense RNA viruses, are characterized by club-like spikes that project from their surface, an unusually large RNA genome, and a unique replication strategy. Roots of Asphodelus microcarpus- which planted in Marsa Matroh Governorate El-Dabaa region is the main food of Spalax leucodon Egyptiacus which showed immunity to many diseases. This plant was mainly reported to have anthraquinone and its derivatives. Asphodelus microcarpus tuber was extracted using methanol 80% that can extract polar, semi-polar, and non-polar compounds. Subsequently the methanolic extract was fractionated to, ethyl acetate. The present work showed that significant antiviral activity of A.microcarpus against coronavirus 229E, further studies are required to emphasizes this effect.

Keywords: Asphodelus microcarpus, Coronavirus 229E, Cytotoxicity, Spalax leucodon Egyptiacus

Introduction

Respiratory viruses are responsible for more deaths globally than any other infectious agent. Animal coronaviruses that “host jump” to humans result in severe infections with high mortality, such as severe acute respiratory syndrome(SARS) and, more recently, Middle East respiratory syndrome (MERS).

The use of plants for therapeutic purposes, such as the treatment of fungi, bacteria and viruses, as well as the treatment of cancerous tumors, is not new and this appears in the papyri and Pharaonic temples. And the matter has evolved and reached the point that many drugs depend heavily on medicinal plants (**Neven, 2010**).

The importance of plants is due to the active substances and secondary plant metabolites in one or more parts of these plants contained in them such as alkaloids, glycosides, corticosteroids, essential oils, etc. These components have biological activity effects (**Aysegul Peksel et al., 2013**).

Anthraquinones are the group of compounds from multiple folk medicines which are utilized in Ayurvedic system of medicines and Traditional Chinese Medicines for the management of various infectious and non-infectious diseases. Further, anthraquinone derivatives are also reported for anti-viral property, anti-inflammatory efficacy, and as immune booster. (**Pukar Khanal et al., 2020**).

Anthraquinones are a class of natural compounds that consist of several hundred compounds that differ in the nature and positions of substituent groups (**Schripsema et al., 1999**). This class of compounds contains derivatives that consist of the basic structure of a 9, 10-anthraquinone moiety (**Bajaj and Ishimaru, 1999**).

The Asphodelus plant contains a group of important secondary components, including flavonoids, phenols, and coumarin. Asphodelus microcarpus contains phenols, the most important of which is the anthraquinone, an important compound through its effects on inflammation, Alzheimer's ,antiviral, and cancerous tumors (**Amalia Petrillo et al., 2017**).

Nutrition is fundamental for survival historical evidence points to the linkage between malnutrition and infection. This association has been confirmed by recent epidemiologic studies and may be due, in part, to the important determinant of host resistance and immunocompetence can be used as an afunctional predictor of outcome this selective review highlights the established links between nutrition, immunity, and illness, and points to some clinical applications of basic knowledge in this area. (**Chandra, 1990; Ayse Biçer, 2020**).

The present study aims to find antiviral effect of the food of Spalax leucodon Egyptiacus (**Fig. 1**) Asphodelus microcarpus (**Fig. 3**) on low pathogenic coronavirus 229E. this sudy the first one examined this effect. This may be useful for another researcher in biological, pharmacological.

Fig. 1. Spalax leucodon Egyptiacus

Fig. 2. Location of plant A.microcarpus

Materials and methods

Plant collection

Asphodelus microcarpus Salzm. et Vivi (Asphodelaceae) is a stout robust herb with roots of several spindle-shaped tubers, widely distributed over the coastal Mediterranean region (**Fig. 2 & Fig. 3**).

The tubers of A. microcarpus were collected from an area 70 km West of Marsa Matrouh, Egypt, during February 2021. The plant was authenticated by Dr. Mohamed El Azzazy, Professor of Plant environmental, El Sadat city University, Egypt. A voucher specimen (AM 21) has been deposited in department Surveying of Natural Resources in Environmental Systems.

Sample preparation and extraction:

The plant material was cut into small pieces using a knife then mixed with 2.5L of 80% methanol. The mixture was allowed to macerate for 24 hours with frequent shaking before being filtered on cellulose filter paper.

Fig. 3. Asphodelus microcarpus

The process was repreated 3 times till exhaustion of the plant material. The filtrate was collected and concentrated under vacuum at 40 °C till reaching 1L volume which was successively partitioned with n-hexane (500 mL X3), dichloromethane (500 mL X4), and ethyl acetate (500 mL X6) to yield the corresponding extracts 0.21 g, 1.95 g and 5.21 g, respectively. During the partitioning process, a yellowish red precipitate was formed that did not dissolve in the remaining aquoues portion. It was seperated and it weighed 7.68g. It dissolved well in methanol.

Antiviral assay

Nawah-Scientific, Egypt, provided the Low Pathogenic Corona Virus (229E) and Vero E6 cells. Vero E6 cells were grown in DMEM medium supplemented with 10% fetal bovine serum and 0.1% antibiotic/antimycotic solution. Gibco BRL provided the antibiotic & antimycotic solution, trypsin-EDTA, fetal bovine serum,

and DMEM medium (Grand Island, NY, USA). The Crystal violet method was used to evaluate antiviral activity and cytotoxicity assays using the recently reported for the cytopathic (CPE) inhibition effect (**Choi et al., 2009; Donalisio et al., 2013**). In brief, Vero E6 cells were seeded into a 96-well culture plate at a density of 2x104cells/well one day before infection. The culture medium was removed the next day, and the cells were washed with phosphate-buffered saline. The infectivity of Low Pathogenic Corona Virus (229E) was determined using the crystal violet method, which monitored CPE and allowed the percentage of cell viability to be calculated. 0.1 mL of diluted virus suspension of 229E containing CCID50 ($1.0 * 10^6$) of virus stock was added to mammalian cells. This dose was selected to produce the desired CPEs two days after infection. For compound treatments, 0.01 mL of medium containing the desired compound concentration was added to the cells. Each test sample's antiviral activity was determined using a 10-fold diluted concentration range of 0.1-1000 µg/ml. The virus controls (virus-infected, nondrug-treated cells) and cell controls (non-infected, nondrug treated cells). For 72 hrs., culture plates were incubated at 37oC in 5% CO2. The development of cytopathic effect was monitored by light microscopy. Following a PBS wash, the cell monolayers were fixed and stained with a 0.03% crystal violet solution in 2% ethanol and 10% formalin. After washing and drying the optical density of individual wells was quantified spectrophotometrically at 570/630 nm. The percentage of antiviral activities of the test's compounds were calculated according to **Pauwels et al. (1988)** using the following equation: antiviral activity= [(mean optical density of cell controls−mean optical density of virus controls)/ (optical density of test−mean optical density of virus controls)] ×100%. Based on these results, the 50% CPE inhibitory dose (IC50) was calculated. Before this assay, we assessed the cytotoxicity; cells were seeded at a density of 2x104 cells/well in a 96-well culture plate. The next day, the culture medium containing serially diluted samples was added to the cells and incubated for 72 hours before being removed and the cells washed with PBS. The following steps were carried out in the same manner as described above for the antiviral activity assay.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 5 (**Graph Pad Inc., La Jolla, CA, USA**). Student t-test was used to compare the means of two independent groups. Statistical significance was designated as $P < 0.05$.

Results and Discussion

Various plants have been used in medicine since ancient times and are known for their strong therapeutic effect. In traditional medicine, diseases of possible viral origin have been treated by many of these plants (**Arik Dahan et al., 2019**).

In this study, Asphodelus microcarpus tuber was extracted using methanol 80 % that can extract polar, semi-polar, and nonpolar compounds. Subsequently, the methanolic extract was fractionated gradually with dichloromethane, ethyl acetate, n-hexane, and red precipitate, resulting in four fractions. These fractions were dichloromethane fraction, ethyl acetate fraction, n-hexane fraction, and a precipitat

According to Thin-layer chromatography (TLC), we found that the EtOAc fraction have amount of anthraquinones so we chose it as mainly fraction which we tested the effect of our plant extract on human melanoma A375, and another one is the precipitate (PPT) which precipitates after fractionation we consider it as a fraction. Observation of cytopathic effects (CPE) induced by virus infection is a practical method to determine the prsence of viruses in the clinical specimens **(Fig. 4)** (**Ting-En Wang ID et al., 2020**).

Fig .5 & **Fig. 6** showed that the value of cytotoxicity in fraction ethyl acetate and fraction precipitate PPT: IC50=4.27ug/ml.

The results of the 50% cytotoxic concentrations (CC50) and the 50% inhibitory concentration (IC50) were determined using GraphPad PRISM Software (Graph-Pad Software, San Diego, USA). Graphs of Cytotoxicity Concentration 50 (CC50) and the 50% inhibitory concentration (IC50) on Vero E6 cells and 229E are presented in **Table 1**.

In-vitro susceptibility of viruses to antiviral agents is normally measured as the inhibitory concentration 50% (IC50), that is the concentration of antiviral that lowers the virus-induced cytopathic effect (CPE) and the number of plaques formed by a given inoculum, by 50 % (**Manuel Cotareloa et al., 1999**).

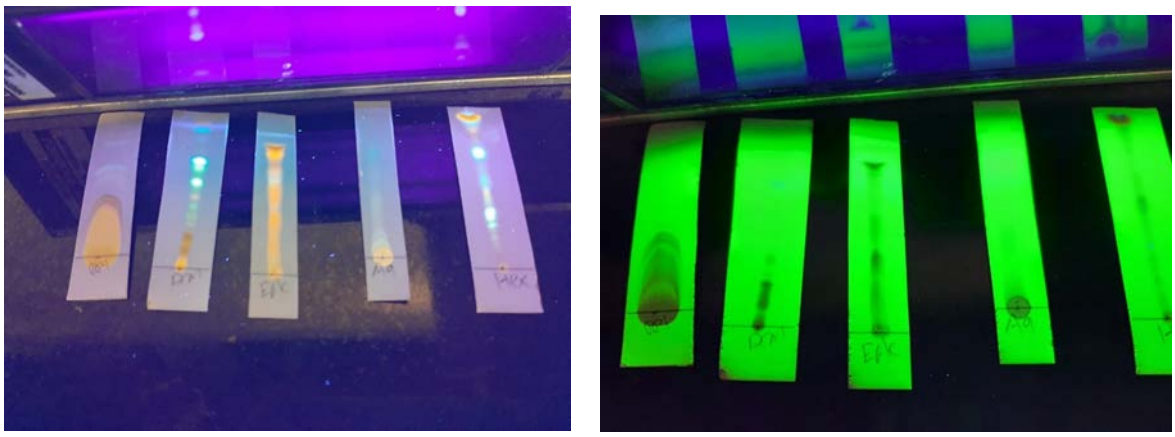
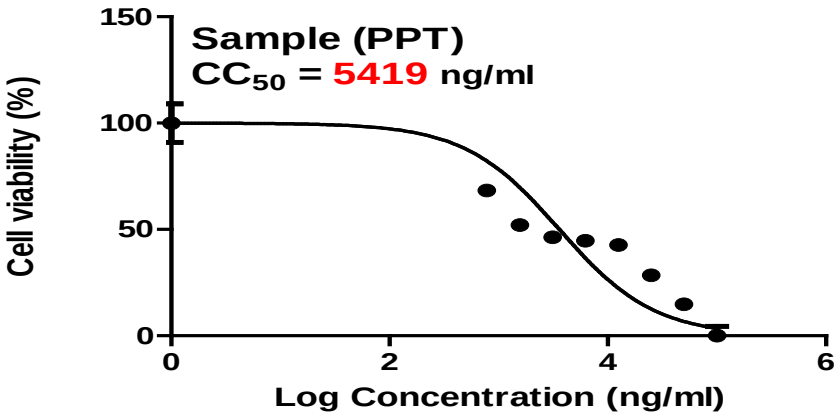


Fig. 4. TLC profile of methanolic extract, dichloromethane fraction, ethyl acetate fraction, n-hexane fraction, precipitate. Infrared spectroscopy was carried out on a Varian 800 FTIR Scimitar series utilizing a PIKE MIracle™ cell with KBr loaded lenses.



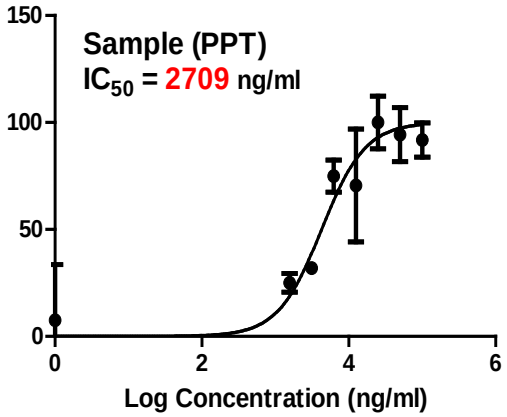


Fig. 5. Sample 1 (PPT).

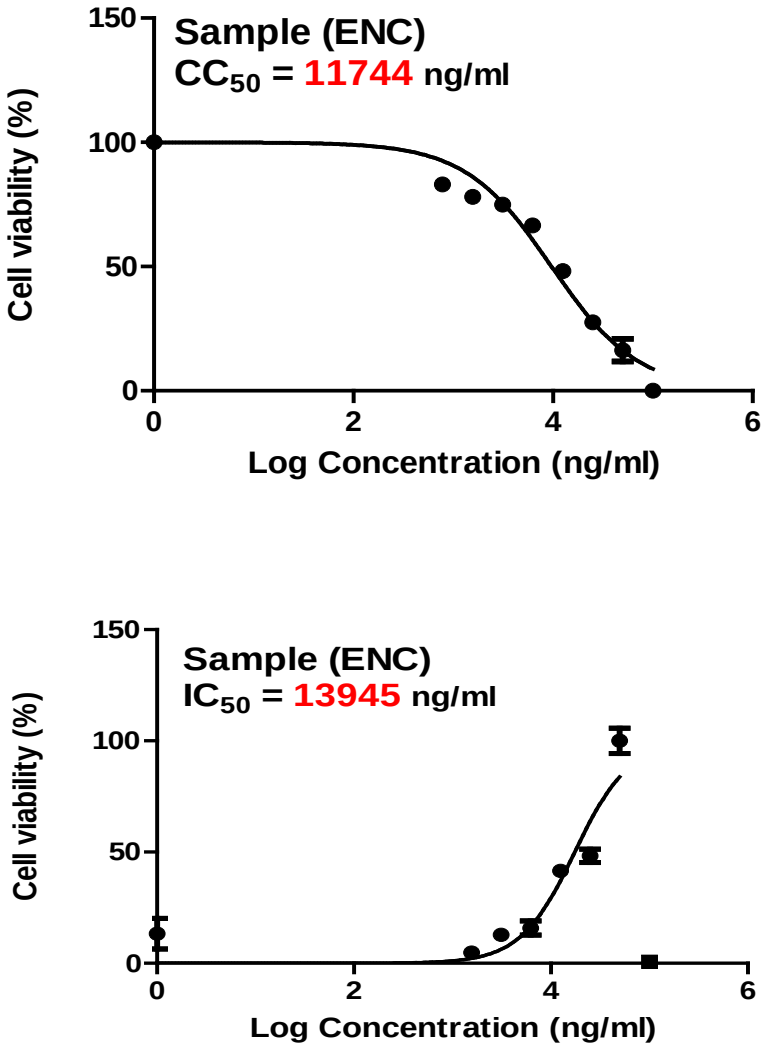


Fig. 6. Sample 2 (EAC).

Table 1. showed the value of CC50 & IC50 and SI of the fractions of A.microcarpus on low pathogenic coronavirus E229.

FRACTIONS	CC50	IC50	SI	Unit
PPT	5419(5.4ug/mL)	2709(2.7ug/mL)	2.0	ng/ml
EAot	11744(11.7ug/mL)	13945(13.9ug/mL)	0.85	ng/ml

According to the results in the table, the tested sample precipitate (PPT) showed antiviral activity against Low Pathogenic Corona Virus (229E) with selective index = estimated CC50/estimated IC50 = 2.0. While the sample ethyl acetate (EAot) showed no any antiviral activities.

Relatively low of Selective Index (SI) (< 1) means the sample could be toxic and cannot be used as a (herbal) drug. If the calculated SI value is between 1 and 10, re-evaluating using other bio system is advocated for confirmation. Sample that showed a relatively low data of SI (< 1) must be contained toxic component. A combination of chemical profiling (LC HR MS/MS) and MVA can be applied to determine the toxic compounds a number of medicinal plants have been reported to contain compounds possessing strong antiviral activity, in previous works, phenolic acids and flavonol glycosides were identified in plants, the better inhibitions against the enveloped virus (HCoV 229E) than the nonenveloped viruses (PV 1, HPeV 1, HPeV 3, and Echo 11) suggest that the active components might inhibit the interaction between the binding sites of the virus to the host cells by inhibiting a ligand of the viral envelope, the interaction between the virus envelope and the extracts could be related to the binding of phytochemical phenolic compounds with the protein coat of the virus and the viral attachment to the host cells (**Inès Thabti et al., 2020**).

Antiviral activity is in general due to anthraquinones., several individual compounds involved in antiviral activity were identified such anthraquinone (**Pius Mpiana et al., 2020**) Anthraquinones could be considered as an immune booster and anti-viral against novel coronavirus (**Pukar Khanal et al., 2020**).

The SI of the sample can be increased by removing the toxic compound(s) using certain chemical method applied acidified water to remove the toxic alkaloids from the leaves of Justicia gendarussa, before performing the extraction; using this method free toxic alkaloids extracts can be prepared (**Gunawan Indrayanto et al., 2021**).

Conclusion

This study is the first study that discusses the effect of A.microcarpus on the Coronavirus 229E, and it clear that there is an effect on it from fraction PPT precipitate.

The presence of an effect on the coronavirus 229E from A.microcarpus, which is the main food for Spalax leucodon Egyptiacus, encourages many studies to pay attention to this plant in the field of nutrition and immunity.

The low percentage of SI can be dealt with through the work of LC MS and identifying the existing components that have an effective effect on the virus, which benefit research and studies of immunity and pharmacology.

References

Anthony, R., Fehr and Stanley Perlman. 2015. Coronaviruses: An Overview of Their Replicationand Pathogenesis Coronavirus Introduction, Methods Mol Biol. 2015; 1282:1-23.

Aysegul Peksel, Sema Imamoglu, Nilay Altas Kiymaz & Nurdagul Orhan. 2013. Antioxidant and radical scavenging activities of Asphodelus aestivus Brot. extracts, *International Journal of Food Properties*, 16:1339–1350.

Di Petrillo, A., Ana Maria González-Paramás, Benedetta Era, Rosaria Medda, Francesca Pintus, Celestino Santos-Buelga and Antonella Fais. 2016. Tyrosinase inhibition and antioxidant properties of Asphodelus microcarpus extracts Di Petrillo et al. BMC Complementary and Alternative Medicine, 16:453.

Gunawan Indrayanto, Galih Satrio Putra, FaridaSuhud .2021 Chapter Six - Validation of in-vitro bioassay methods: Application in herbal drug research Volume 46:273-307.

Hosny Abd El-Fattah. 1997. Chemistry of Asphodelus fistulosus International Journal of Pharmacognosy, 35:4, 274-277.

Khiralla, A., Spina, R., Varbanov, V. 2020. Evaluation of Antiviral, Antibacterial and Antiproliferative Activities of the Endophytic Fungus Curvularia papendorfii, and Isolation of a New Polyhydroxyacid. Microorganisms, 8, 1353; doi: 10.3390/microorganisms8091353.

Mohammed M. Ghoneim, Khaled M. Elokely, Atef A. El-Hela, Abd Elsalam I. Mohammad, Melissa Jacob, Stephen J. Cutler,Robert J. Doerksen, and Samir A. Ross .2014. Isolation and characterization of new secondary metabolites from *Asphodelus microcarpus* Med Chem Res. 2014 July, 23(7).

Nevine Abd El-Gawad Ali Hasan. 2017. Medicine in ancient Egypt Egypt J Intern Med The Egyptian Journal of Internal Medicine, 1110-7782,29:33–34, 2017.

Pukar Khanal · B. M. Patil · Jagdish Chand · Yasmin Naaz .2020. Anthraquinone Derivatives as an Immune Booster and their Therapeutic Option against COVID19, Natural Products and Bioprospecting, 10:325–335.

STATE-OF-ART, Interaction of C. forskohlii with P.indica for Secondary Metabolites Evaluation.

Ting-En WangID, Tai-Ling Chao, Hsin-Tsuen Tsai, Pi-Han Lin, Yen-Lung TsaiID, SuiYuan Chang. 2020. Differentiation of Cytopathic Effects (CPE) induced by influenza virus infection using deep Convolutional Neural Networks (CNN), PLOS Computational Biology. https://doi.org/10.1371/journal.pcbi.1007883.

Yejin Jang, Jin Soo Shin, Joo-Youn Lee, et al. 2020. In Vitro and in Vivo Antiviral Activity of Nylidrin by Targeting the Hemagglutinin 2-Mediated Membrane Fusion of Influenza a Virus. Viruses, 12, 581; doi: 10.3390/v12050581.