

## Antiviral Activity of Some Herbs against *Polio Virus in Vitro*

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### ABSTRACT

**Back ground:** Recent studies about plants and botanical components used for different diseases and disorders as well as tradition herbal medicine had become the focus of attention of researchers and specialists in medicine. **Objective:** The current study aimed to investigate the antiviral activity of star anise seeds and black seeds aqueous extracts against *Polio virus* in L20B and Rd cell lines culture for understanding herbs extracts antiviral activity towards RNA covid 19 virus.

**Material and Methods:** Applying two methods of *in vitro* treatment, pre-treatment method and simultaneous treatment either by treating the infected cell with *Polio virus* suspended in 2 ml culture tubes or treating the infected cells with *Polio virus* in a microtiter plated of 96 flat bottoms wells. **Results:** The antiviral activity against *Polio virus* was improved through simulations treated of infected cells with aqueous extract of star Anise seeds and Black seeds *in vitro* even the later gave the more potent effect. **Conclusion:** Different biological activates might be attributed to the differences in the active secondary metabolites constituent in plants extract made estimation for their antiviral activity vary from each other.

Keywords: *Illicium verum* seeds, *Nigella sativa*, Black seeds, *POLIO VIRUS*, Antiviral activity.

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### 1-INTRODUCTION

Both conventional medicine and traditional Chinese medicine (TCM) succeeded in curing patients with infection of SARS-CoV-2 in China (1). This clinical guideline in China besides; the huge experiences in TCM for the reduction in different respiratory bacterial and viral infectious as in Middle East Respiratory Syndrome (MERS) and SARS disorders with the correlation in the absence of a specific antiviral therapeutics and vaccine at that time, for all these reasons and others, the need for investigation an effective medicinal plant, herbs and their components has become one of the quick steps that must be taken to save such a predators. In addition, the genetic similarity with epidemiology and pathogenesis homology between the SARS-CoV-2 and SARS-CoV19, lead the way to the use of TCM in the treatment of coronaviral infections (2). COVID-19, the viral disease has quickly resulted in a pandemic and is a great threat to global public health, the development of new drugs, such as natural herbal drugs, has gained attention and become a rush that mobilized researchers from all areas across the world, among these, natural products that can be important resources in the development of COVID-19 treatment, as they have already contributed in the past to treatments against other viruses, such as HIV, MERS-CoV, and influenza, *Illicium verum* the Chinese star anise and *Nigella sativa* the black seeds. These natural herbs are described in the long term as they contain phytochemical bioactive substances such as flavonoids, polyphenols, and terpenes that have traditionally been used in flu and cold as antiviral (3). *Illicium verum* (known as Chinese star anise) Schisandraceae family is known to treat viral infections (3) *Nigella sativa* (black seeds, NS) has been traditionally used as a complementary drug in many Arabian countries. Studies viewed that NS had many pharmacological actions which include: anti-tumor (4), anti-diabetic (5), anti-inflammatory (6), analgesic (7), anti-convulsion (8), anti-bacterial (9), reduction in lipid level (10), anti-asthmatic (11) and anti-hypertensive properties (12). Its mode of action is mediated by anti-oxidant, immunomodulation (13), cytoprotective, and inhibitory effects on mediators of inflammation (14).

Development of antiviral treatment is difficult, as it depends on the knowledge of its pathobiology; also it is difficult to find drugs that do not cause strong side effects. For this reason, a general antiviral assay protocol has been employed which requires knowledge about viral molecular structure, inoculation, and replication mechanism,

and has been developed to estimate that the drugs can combat the capability of the virus. The development of drug resistance is another challenge to be faced, as it increases the spreading of a pandemic disease.

As there is a legal restriction on Iraq dealing with goods supplying labs for viruses research, and since the good and save laboratory performers require for viral work to get a stage 4 requirement including lab safety; therefore, no available work on Covid 19 virus has been allowed. Thus in the present study, the RNA *Polio virus* was employed instead of the Covid-19 virus as both viruses have genetic homology. The current study aimed to investigate the antiviral activity of star anise seeds and black seeds aqueous extracts in vitro against *Poliovirus* as a simulation study for understanding herbs extracts' antiviral activity towards RNA COVID-19 virus.

## 2- MATERIAL AND METHODS

### Plant Collection and Aqueous Extraction (15)

Seeds of both plants *Illicium verum* and *Nigella sativa* were collected from a local market. About 50g from both seeds and extracted by digested method separately with 500 ml distilled water at 60 °C for 2 hours with the aid of a magnetic stirrer for continuous shaking. Then, it is cooled and filtered to be evaporated by a rotary evaporator to dryness. About 2gm from star anise residue was re-dissolved to prepare a stock solution of 40mg/ml distilled water (A solution). Black seed residue was re-dissolved in distilled water to get a stock solution of 100mg/ml concentration (B solution). Both solutions were sterile through a 0.22mm Millipore filter.

### Antiviral Activity of the plant Extract (16, 17)

This work was held in Central public health laboratories/ Baghdad / Iraq. Two types of cell suspensions were used in this study, the RD and L20B cell lines. The antiviral protocol employed in this study in two manners.

#### a- Treatment of the infected cell with *Poliovirus* suspended in 2 ml culture tubes.

#### b- Treatment of the infected cells with *Polivirus* in a 96 microtiter plate flat bottom wells.

In both treatment processes the application included the antiviral effect of the plant extract in two methods:-

*i*-The first method, pre-treatment method: The plant extract and virus suspensions were incubated together for 1 hour at 4°C, and then the cultured cells were inoculated with the mixture of (virus + extract) and incubated for 1 hour at 37°C.

*ii*-The second method included simultaneous treatment of the cells inoculated with virus suspension and then the extracts were added. The plates were incubated at 37°C in a humidified atmosphere and 5% CO<sub>2</sub>.

This protocol was according to references (16 and 17) with some modifications.

#### a- Treatment of the infected cell with *Polio virus* in 2ml culture tubes.

The application process was done in pre- and simultaneous treatment with plant extract in tubes containing 2 ml RD or L20 B cells suspension, to be inoculated with 20µl virus suspension and 20µl plant extract solution (A and B), separately and the cells were examined daily using an inverted optical microscope.

#### b- Treatment of the infected cells with the *Poliovirus* in a microtiter plate of 96 flat bottom wells.

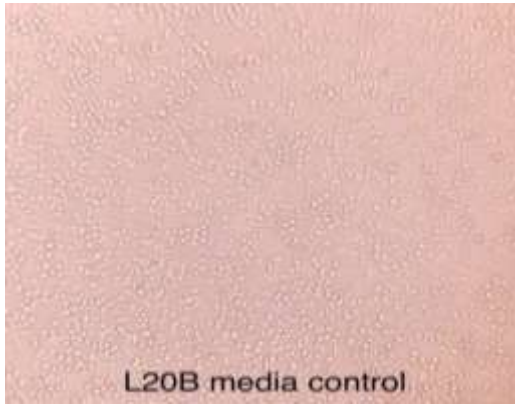
The seeded plates with 100 µl RD or L20B cell suspension were applied with both methods of treatment (pre and simultaneous). Confluent monolayers of each cell line in separated 96-well tissue culture plates were obtained to be treated and then incubated at 37°C with 5% CO<sub>2</sub>; for 24 hours. Positive control contained cells inoculated with the virus without the extracts, while negative control wells included only cells suspended in the medium. The MTT dye(5 mg/ml PBS) solution(3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma) was added to each well, and the plates were further incubated at 37°C for 4 hours. The medium was removed and 100 µl of dimethyl sulfoxide (DMSO) was added to each well. Plates were shaken in a microplate shaker for 5 minutes, and the absorbance was read at a wavelength of 570nm using a microtiter plate reader. The experiments were performed in duplicate and repeated three times.

Virus inhibitory rate % ((extract + virus – virus control)/(cell control– virus control)) X 100.(17)

### 3-RESULTS

#### Antiviral Activity of the Plant Extract

a- Treatment of the infected cell with *poliovirus* in 2 ml suspended cell culture tubes for L20B and RD cells



Figure(1): L20B cells control without treatment



Figure(2):L20B with Extract A(+ve control)



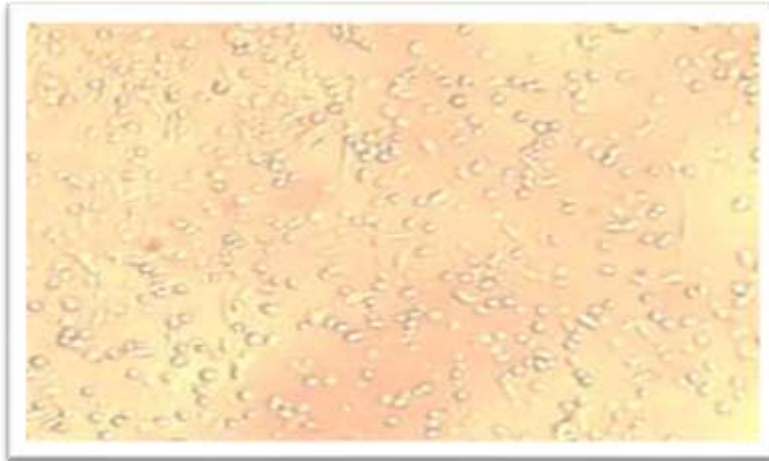
Figure(3): L20B with Extract B(+ve control)



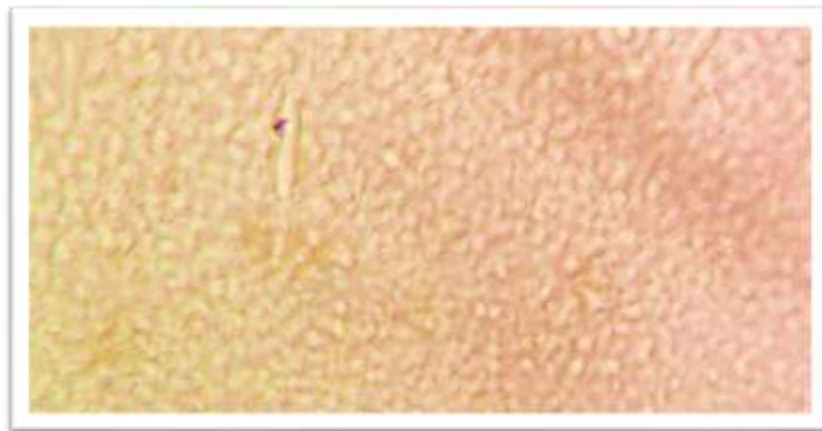
Figure(4):L20B cells inoculated with virus only



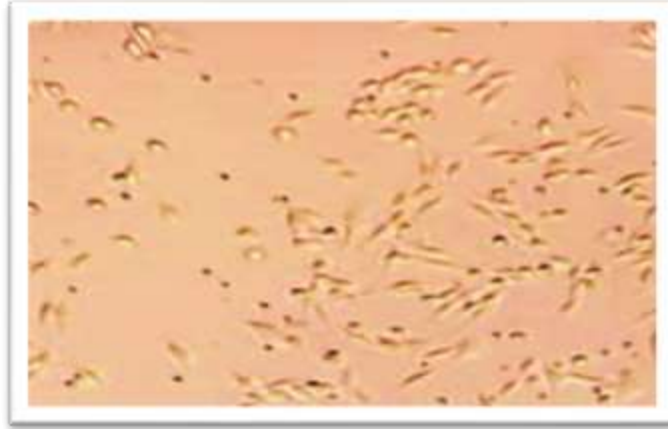
Figure(5):The pre-treatment of virus suspensions Incubated with plant extract A for L20B cell culture



Figure(6):The simultaneous treatment of L20B cells inoculated with virus suspension and plant extract A



Figure(7):The simultaneous treatment of L20B cells inoculated with virus suspension and plant extract B



Figure(8):The pre-treatment of the cells inoculated with virus suspension and plant extract B



Figure(9):RD cells control without treatment as -ve control



Figure(10): RD cells with ExtractA (+ve control)



Figure(11): RD cells with Extract B(+ve control)



Figure(12): RD cells with virus only



Figure(13): The simultaneous treatment of RD cells inoculated with virus suspension and plant extract A



Figure(14):The pre-treatment of the cells inoculated with virus suspension and plant extract A

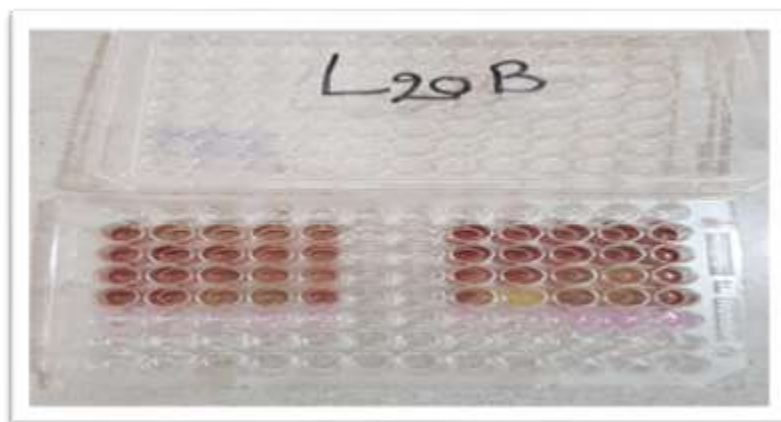


Figure(15):The simultaneous treatment of RD cells inoculated with virus suspension and plant extract B

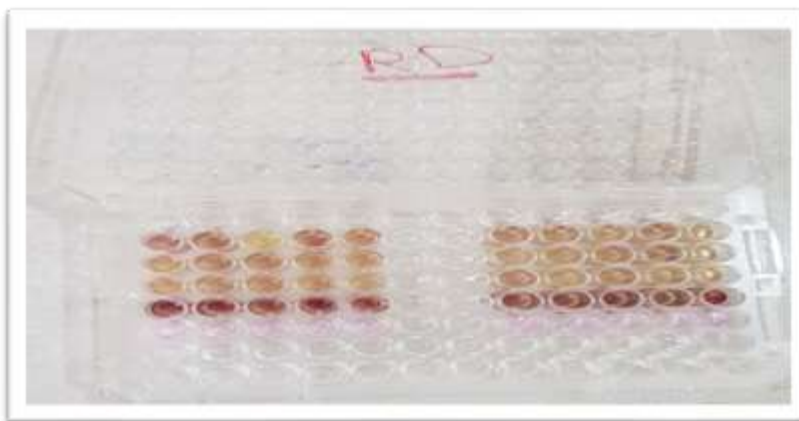


Figure(16):The pre-treatment of virus suspensions incubated together with B plant extract outside of RD cell culture for 1 hour at 4°C

b- Treatment of the infected cells with the *polio* virus in a microtiter plate of 96 flat-bottom wells



Figure(17): The MTT assay for the L20B cell lines for 24 hours with both plant extracts and virus inoculation



Figure(18): The MTT assay for the RD cell lines for 24 hours with both plant extracts And virus inoculation

Table (1): The antiviral effect as percentage for both plant extracts in two ways treatments for L20B and RD cells

<b>Extract</b>	<b>Antiviral effect% L20B cell line</b>	<b>Antiviral effect% RD cell line</b>
pre-treatment with <b>A extract</b>	60	51
simultaneous treatment with <b>A extract</b>	83	91
pre-treatment with <b>B extract</b>	40	36
simultaneous treatment with <b>B extract</b>	92	90

#### 4-DISCUSSION

As shown in Table-1- the potent antiviral effect was for B extract of the black seeds( *Nigella sativa seeds*) when the plant extract was applied as simultaneous treatment with viral culture in both cell line cultures L20B & Rd cell lines. In the pre-treatment experiment, solution (A) star anise, or *Illicium verum* seeds appeared to possess more antiviral activity in both cell lines. These different biological activities might be attributed to the differences in the active secondary metabolites constituent in each extract which may needed to make an estimation for these bioactive components and study their activity, along with the real mechanism which is still unknown.

The similarity between SARS-CoV-1 and Covid-19 also brings light to the development of new drugs or even vaccines. Great hope has emerged from the possible anti-SARS-CoV-2 activity of some plants. As most of the studies are theoretical or do not present analytical validation, and the real mechanism for antiviral activity is still



unknown, a long path is still ahead in terms of biological analysis and optimized extraction and production, hat the systematic evaluation intends to reinforce the effort of this work.

## 5-CONCLUSION

Studies that involve different epidemic viral infections have been of great interest to researchers recently(18,19) and the attempts for new treatments epically from herbal sources and even new vaccinations strategies have been considered one of the crucial components of disease preventions towards these emerging virus infection(20). Thus the current study lightened the effeteness rule of herbs in management many viruses emerged or re-emerged from the obscurity and became considerable threats to the global health.

## 6-ACKNOWLEDGMENTS

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### الفعالية مضادة للفايروسات لبعض الاعشاب ضد فايروس شلل الاطفال في المختبر

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#### الخلاصة

**خلفية الموضوع:** أصبحت الدراسات الحديثة حول النباتات والمكونات النباتية المستخدمة في الأمراض والاضطرابات المختلفة وكذلك الطب العشبي التقليدي محط اهتمام الباحثين والمختصين في الطب. **الهدف من الدراسة:** هدفت الدراسة الحالية إلى التحقيق في النشاط المضاد للفايروسات للمستخلصات المائية لبذور اليانسون النجمي ولبذور الحبة السوداء ضد فايروس شلل الأطفال في مزارع خطوط الخلايا Rd وL20B لفهم نشاط مستخلصات الأعشاب المضادة للفايروسات تجاه فايروس RNA covid 19 . **المواد وطرق العمل:** تم العمل خلال تطبيق طريقتين من العلاج في المختبر ، طريقة العلاج المسبق وطريقة العلاج المتزامن وفي كلتا الطريقتين يكون نمط العلاج إما عن طريق معالجة الخلايا المصابة بفايروس شلل الأطفال والمعلقة في أنابيب تحوي 2 مل من المعلق الزراعي والخلايا أو معالجة الخلايا المصابة بفايروس شلل الأطفال في اوعية الزرع النسيجي ذو- 96 حفرة مسطحة القاع. **النتائج:** تم تأكيد النشاط المضاد للفايروسات ضد فايروس شلل الأطفال من خلال المعالجة المتزامنة للخلايا المصابة بالمستخلص المائي لبذور اليانسون النجمي والبذور السوداء في المختبر حيث أعطى هذا النوع من العلاج التأثير الأكثر فاعلية ضد النشاط الفايروسي. **الاستنتاج:** قد تعزى النشاطات البيولوجية المختلفة إلى الاختلافات في المكونات الفعالة المتواجدة في مستخلصات النباتات، مما يجعل تقدير نشاطها المضاد للفايروسات يختلف عن بعضها البعض.

**الكلمات المفتاحية:** بذور *Illicium verum*، حبة البركة، حبة السوداء، فايروس شلل الأطفال، نشاط مضاد للفايروسات.