

In Ovo Antiviral Screening of Cholistani Plants Against Swine Influenza Virus and Confirmation Through Real Time PCR

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Abstract

Cholistani plants are a rich source of many biological/pharmacological agents. Recent studies have supported the idea that cholistani plants are rich in antimicrobial agents especially against viruses. Medicinal plants like *Haloxylon recurvum*, *Salsola baryosma*, and *Achyranthes aspera* hold the potential to control viruses like Avian Influenza and Swine Influenza. Prevention by vaccination is the best treatment so far, but some side effects are associated with vaccines alone or in combination with other influenza virus strains. Eleven different Cholistani plants (*H. recurvum*, *H. salicornicum*, *A. aspera*, *Suaeda fruticosa*, *Sporobolus icolados*, *Solanum surattense*, *Ochthochloa compressa*, *Neurada procumbens*, *Panicum antidotal*, *Oxystelma esculentum*, and *Salsola baryosma*) were selected and tested against SI strain H1N1. The samples were washed and dried under shade at RT. All the plants were ground and used in making extracts using different solvents. The SIV H1N1 isolate was confirmed through real-time (rt) PCR and then subjected to 9-11 days old chick embryonated eggs. Eggs were candled and viability of embryo was confirmed. After 48hrs, allantoic fluids were collected, and viral loads were also confirmed through HA test and RT-PCR. Most of the extracts were found effective in controlling growth of the virus. Among these, n-hexane and ethanol (EtOH) extracts were found most effective, and all extracts were active against SIV H1N1. In the case of ethyl acetate and methanol (MtOH) 9 out of 11, similarly, in n-butanol (BtOH), 7 out of 11 extracts were active against SIV H1N1. All the results were first confirmed HA test and later through rt-PCR. The virus was not detected in the outcomes of rt-PCR, confirming the antiviral potentials of extracts of Cholistani plants. This study strongly supported the idea that Cholistani plants are a rich source of antiviral agents and can be used in pure and crude form.

Key Words: Antiviral activity, Cholistani Plants, Swine Influenza, RTPCR

1. Introduction

Swine influenza virus (SIV) strain H1N1 is a widespread pathogenic strain in the swine herds. In the last century influenza virus has been considered a causative agent in 1918, 1957, 1968 for the Spanish, Asian, and Hong Kong Flu, respectively (Rewar, 2015). Furthermore, SIV H1N1 is also related to the descendant's strain that prompted the 1918 flu epidemics. Moreover, through the 20th century, the descendants of this virus also spreading in humans, contributing to the classical flu of seasonal epidemics. Now, about 60% of the world's pigs are produced in Asia, and over 40% of pigs have been associated

with China alone, and due to this reason, the chances of the viral outbreak were also increased. It is a definitive cause for the survival of the swine virus, leading to higher chances for the interaction between the pigs, humans, poultry flocks, and the wild birds of Asia (Lemey *et al.*, 2014). It has been generally observed that pigs are the prominent transitional host in developing avian viruses to mammalian hosts due to interspecies transfer (Fragaszy *et al.*, 2016). In 2009, H₁N₁ arose from pigs inflicting a preliminary outbreak in Mexico and enhancing a worldwide epidemic in a couple of months. Through the first couple of weeks of surveillance, the virus spread globally, affecting over 214 states and bringing about over 18,449 deaths (Cheng *et al.*, 2012). On 13th August 2009, WHO recommended 1 82,166 research laboratory-confirmed cases of H₁N₁ and 1799 were expiries from 178 states. In India, from 2015-2016, a significant outbreak of H₁N₁ have killed more than 1,900 people. In 2016, several infections and deaths due to SIV were reported. In 2017 there was a revival of this disease. H1N1 outbreak started in January 2017 resulted in 22 186 infections. By keeping in view, WHO raised its pandemic alert level to 5 and increased the demand of antiviral compounds for the medication of this diseases (Lin *et al.*, 2017).

Swine influenza virus

SIV is a single-stranded RNA virus belongs to orthomyxoviridae family (Dayem *et al.*). It has four genera named A, B, C, and D (Li *et al.*, 2016). Among all the types of SIV, only three subtypes, H1N1, H1N2, and H3N2, are responsible for human influenza (Chiapponi *et al.*, 2018). Critical medical conditions of SI include fever, high breathing rates, including cough, strolling nostril, and sore throat. Headache, bodyache, fatigue, diarrhea, and vomiting have also been noticed. Old age individuals with chronic clinical conditions have a higher chance of infection and severity of the fundamental conditions (Bhiari *et al.*, 2019). The important factors involved in SIV transmission is inhalation or ingestion of droplets containing virus produced through sneezing or coughing by infected individual (Kaur & Kaushik, 2018). It is most likely to be expected that 3 to 5 million cases of H1N1 arise globally every year, and ¼ - ½ million deaths occur due to this disease (Nandy *et al.*, 2018). For regular screening commercially available kits should be used for SIV testing. Now a day's latest techniques, *e.g.* genetic screening through real-time PCR is highly recommended by WHO (Jhunget *et al.*, 2013).

Cholistani plants with medicinal significance

Medicinal plants are an essential part of herbal as well as modern medicines. Modern medicines are still depends on natural sources for new templates. Nowadays, use of plant-based drugs are very common in different parts of the world. These drugs are considered effective, cheap, with no or least side effects, and several other motives. The demand for plant-based drugs has been increased manifold in the last twenty years. Recently, many antiviral drugs have been developed by herbal products and used as a substitute source against viral diseases (Ahmed *et al.*, 2014).

Effective antiviral treatment is necessary to prevent and control viral pandemics in humans and livestock. The use of herbal medicinal plants with rare side effects compared to synthetic medications has successfully made them acceptable and famous worldwide. For the screening of antiviral activity, the extracts of Cholistani plants have been used. This screening has prompted the discovery of active inhibitors for *in vitro* viral development and the utilization of the ethno-pharmacological approach to separate new bioactive plant compounds. Numerous local plants have been utilized to treat several human infections and their livestock by the local inhabitants of Cholistan. The key points concerning the research are to discover the uses of the shrubs by local inhabitants, counsel with the specialists of plants with their assessment, and determine the synthetic arrangement of the above-cited plant extracts. The studies support that traditional plants have potential against viral diseases and can be used as a core basis in improving novel antiviral medications in the future. For this reason, there's a need to discover new and

more fantastic effective antiviral drugs for the remedy of various types of viral contagions, so this investigation was focused on plant remedies used in the treatment of cutaneous conditions. The extracts from the plants have been verified as an antidote for various types of viral disorders (Vijayan *et al.*, 2004).

2. Materials and Methods

2.1 Collection of plants

Eleven different fresh plants, *i.e.*, *Haloxylon recurvum* (Khar, Sajji), *Haloxylon salicornicum* (Lana), *Achyranthes aspera* (Puth Kanda), *Suaeda fruticosa* (Kali Lani), *Sporobolus icolados*, *Solanum surattense* (Kandiari, Muhakri), *Ochthochloa compressa* (Chimber), *Neurada procumbens* (Chhapri), *Panicum antidotale* (Murrot, Bansi, ghaa), *Oxystelma esculentum* (Dudhani) and *Salsola baryosma* (Lani) were collected as a whole plant from Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan and nearby area of Lal Suhanra National Park Bahawalpur Punjab, Pakistan. The voucher for each plant was saved. The samples were washed and dried under shade at RT (25°C) for at least ten days.

2.2 Isolation and identification of Swine influenza virus (SIV) H₁N₁

SIV H₁N₁ was isolated from patient-reported at influenza diagnostic facility, Bahawal Victoria Hospital, Bahawalpur. The nasopharyngeal swabs were maintained in DMEM media, and the sample was confirmed through real-time (RT) PCR by strain-specific primers. The medium was filter-sterilized, and the virus was subjected to *Inovo* inoculation in 9-11 days old Chicken embryonated eggs (CEE).

2.3 Preparation of plant extracts

The dried plants were cut into small pieces, crushed via pestle/mortar, and ultimately converted into powdered form by an electric grinder. Then these powders were preserved in sealed containers at RT. The 200ml of each solvent like MtOH, *n*-hexane, EtOH, *n*-butanol, and ethyl-acetate was mixed with 10g of powder from each plant. The solutions were saved in sealed containers for 96 h, with vigorous shaking. Finally, the solutions were exposed to a rotary evaporator at 45-50°C. After evaporation; the concentrates were cleaned with a respective solvent/chloroform mixture and then re-evaporated. Finally, the precipitates were weighted to respective solvent, vortexed, and filter through Whatman filter paper. The samples were sterilized by passing through a syringe filter (0.22 µm) and kept at -20°C (Joshi and Kaur, 2013).

2.4 Virus Inoculation

The 9-11 days old Specific pathogen-free were obtained from Govt poultry farm, Model Town A, Bahawalpur and used for inoculation purposes. SIV was injected in the chorioallantoic fluid of CEE. The eggs were candled before inoculations to check the viability of embryos. The broader ends of eggs were swabbed with 70% alcohol and allow to evaporate. This whole practice was done in Biosafety Cabinet type II. The broader ends of eggs were drilled by hand with a sterilized standard pin and instantly inoculated with viral strain via the chorioallantoic route. The hole was closed with melted wax after the inoculation, and eggs were incubated at 37°C. The viability of eggs was checked daily. The allantoic fluid was collected 48-72 h post-inoculation, and a hemagglutination test was performed to evaluate the titer of the virus. Serial passages were done before taking antiviral trials to take the desired titer of the SIV H₁N₁ virus (Wei *et al.*, 2015).

2.5 Hemagglutination Test

Fresh chicken blood, 5 ml was added to 20 ml Alsever solution (sodium chloride=0.42g, citric acid=0.55g, sodium citrate=0.8g, D-glucose=2.05g, Distilled-H₂O=100ml) in a test tube. The mixture was centrifuged at 4000 rpm for 5 min, and the supernatant was discarded. The packed red blood cells (RBCs)

were washed with 5 ml phosphate buffer saline (PBS) solution, and this step was repeated thrice. 1% RBC solution was made by taking 10 μ l packed RBC into 1 ml PBS solution. Then a round-bottom 96-well plate was taken, and 50 μ l of the PBS solution was added to all the plate wells. In the first well, 50 μ l of the viral strain was added and mixed and then transferred to 50 μ l volume to the next well on its right side (serial dilution), then repeated this mixing till the 11th well and lastly, 50 μ l was then discarded from the final (11th) well. The 12th well was a negative control (PBS solution). 1% of RBC's solution (50 μ l) was transferred to each of the wells and mixed smoothly, and the plate was incubated at 37°C for 1 hour to note down the results (Matusevich *et al.*, 2015; Wang *et al.*, 2018).

2.6 RNA extraction from allantoic fluid

For the RNA extraction, 1ml of allantoic fluid was taken and centrifuged at 1000-3000 rpm. The medium was removed by aspiration, and the cell pellets were resuspended in 1-2 ml of sterilized ice-cold PBS. The cells were harvested by 1000-3000 rpm centrifugation, and the PBS was removed. Then 1ml or 2 ml of solution D (25 mM sodium citrate, 0.1 M β -mercapto EtOH, 4 M guanidinium thiocyanate, and 0.5% (w/v) sodium lauryl sarcosinate) was added. The cells were homogenized for 15-30 seconds at RT. After this, the homogenates were transferred to a fresh polypropylene tube, and 0.1ml of 2 M sodium acetate (pH 4.0), 1ml Phenol, and 0.2ml of chloroform-isoamyl alcohol per ml of solution D were added. The mixtures were vortexed vigorously for ten seconds and incubated on ice for fifteen minutes, and then shifted to the next step centrifugation at 10000 rpm for 20 min at 4°C. The upper aqueous portion comprising extracted RNA was transferred to a new tube. An equivalent volume of isopropanol was mixed in the extracted RNA, and then the solution was vortexed. The RNA was permitted to precipitate for 30-45 min at 4°C. The precipitated RNA was collected using centrifugation at 1000 rpm for 20 min on the ice at 4°C. The isopropanol was removed, and 0.3 ml of solution D was added.

Then the solution was shifted to a microfuge tube and mixed well. The solution was incubated for 30-45 min on the ice at 4°C. Precipitated RNA was collected by centrifugation at 10000 rpm for 10 min on the ice at 4°C. Washing of the pellet with 75% EtOH was done and the centrifugation step repeated. A pipette tip removed the EtOH. After this, open the tube on the bench to allow the EtOH to evaporate. They were dissolved in deionized formamide to store at -20°C (Chomczynski, 1992).

2.7 In ovo antiviral assay

Antiviral estimation was established to analyze antiviral activity from selected Cholistani plants, *in ovo*. The filter pure plant extracts were blended with an equivalent volume of viral inoculums and then inoculated 9-11 days old (CEE) as the process defined by (Rajbhandari *et al.*, 2001). In case of negative control, a Phosphate buffer solution was used, and a virus without any plant extracts was used in case of virus control. All the inoculated eggs were collected after 48-72 h and assessed through hemagglutination test and RT-PCR.

2.8 Real-Time (RT) PCR

RT-PCR was used for quantification of SIV H₁N₁ after In Ovo antiviral assay. PCR master mixture was prepared on ice. The components used to prepare RT-PCR were given; Buffer 12.5 μ L, Forward/reverse PCR primer 400 nM, Enzyme mixture 1 μ L, TaqMan probes 120 nM, RNA sample. The final volume of RT-PCR was 25 μ L per reaction. For the negative controls, nuclease-free water was utilized instead of the sample. The master mix was dispensed to each PCR tube, and the sample was added to each reaction along with positive and negative control. Applied Biosystems® 7500 Real-Time PCR System verified the performance of the kit. The whole reaction was run at the WHO Influenza reference lab (BSL-3 lab) at Nishtar Medical University Multan, and all the lab staff was pre-vaccinated against SIV H₁N₁.

2.9 IC₅₀

The serial dilution method (1:2, 1:4, 1:8) was determined the IC₅₀ of each positive extract. Easy fit software was used to calculate dose and time-dependent curves by linear regression.

3. Results

3.1 Antiviral activity

All the extracts of Cholistani plants selected for their antiviral effect effectively controlled SIV H₁N₁ growth. However, the effect varied from plant to plant and extract to extract. The antiviral effect is reported in terms of HA titers. The higher the HA titer value, the lower the antiviral effect of plant extracts. The *n*-hexane extract of all Cholistani plants has shown the highest antiviral effect revealing an HA titer value ranged between 0 to 32 and log reduction from 9 to 5 (table 1). The plant's *Salsola baryosma*, *Ochthochloa compressa*, *Neurada procumbens* exhibited optimal antiviral activity with 0 HA titer (<log₂), showing almost complete control over viral growth. According to the results of this study, 11 out of 11, *n*-hexane and EtOH extracts, 9 out of 11, ethyl acetate and MtOH extracts, and similarly 7 out of 11 butanol extracts were strongly positive against SIV H₁N₁. The extracts of EtOH, MtOH, *n*-hexane, *n*-butanol, and ethyl acetate of *Haloxylon recurvum*, *Salsola baryosma*, *Haloxylon salicornicum*, *Achyranthes aspera*, *Neurada procumbens*, *Sporobolus icolados*, *Solanum surattense*, *Ochthochloa compressa*, *Suaeda fruticosa*, *Oxystelma esculentum*, and *Panicum antidotale* exhibit suitable antiviral activities against SIV H₁N₁. The HA-based results of *n*-hexane extracts were further confirmed through real-time (RT) PCR.

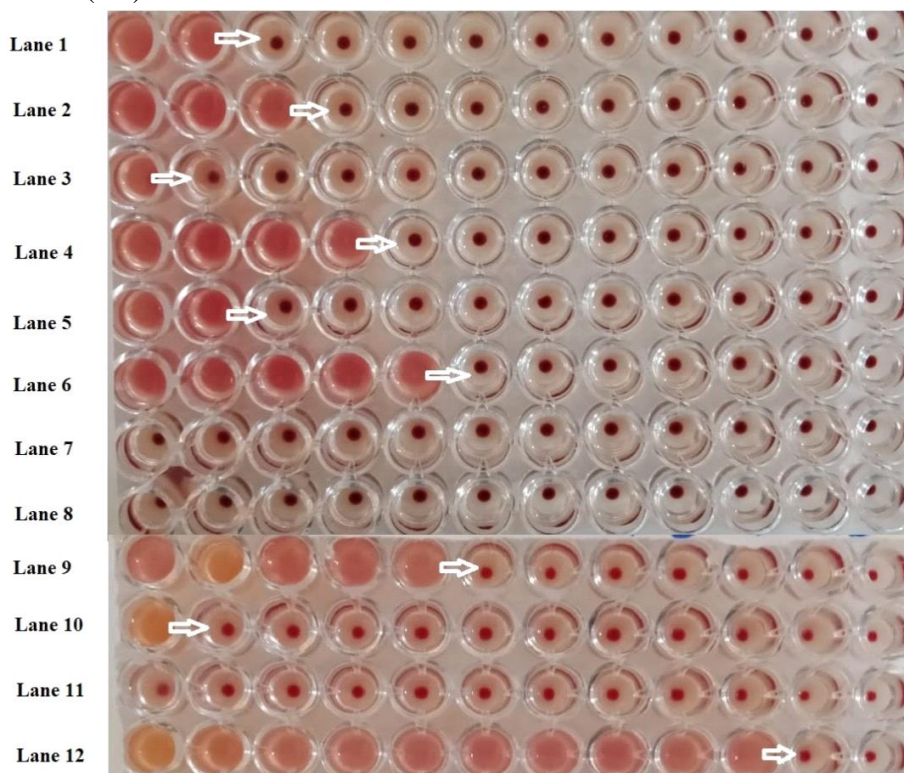


Fig 1. Anti SIV H₁N₁ activity of *n*-hexane extract of different Cholistani plants

Lane 1 shows HA titer of *H. recurvum*, Lane 2 shows HA titer of *H. salicornicum*, Lane 3 shows HA titer of *A. aspera*, Lane 4 shows HA titer of *S. fruticosa*, Lane 5 shows HA titer of *S. icolados*, Lane 6 shows HA titer of *S. surattense*, Lane 7 shows HA titer of *O. compressa*, Lane 8 shows HA titer of *N.*

procumbens, Lane 9 shows HA titer of *P. antidotale*, Lane 10 shows HA titer of *O. esculentum* Lane 11 shows HA titer of *S. baryosma* and Lane 12 shows HA titer of SIV H₁N₁ control.

Table 1: Anti SIV H₁N₁activities of extracts of selected Cholistani plants

Cholistani Plant	EtOH extract		Ethyl- acetate extract		MtOH extract		n-butanol extract		n-hexane extract	
	log ₂ reduct ion of SIV H1N1	*IC ₅₀	log ₂ reductio n of SIV H1N1	IC ₅₀	log ₂ reductio n of SIV H1N1	IC ₅₀	log ₂ reductio n of SIV H1N1	IC ₅₀	log ₂ reductio n of SIV H1N1	IC ₅₀
<i>H. recurvum</i>	**<log ₂	12.5	4	-	9	12.5	<log ₂	3.15	8	25
<i>H. salicornicum</i>	5	50	6	50	8	25	8	25		25
<i>A. aspera</i>	<log ₂	3.12	<log ₂	12.5	2	-	2	-	9	12.5
<i>S. fruticose</i>	6	25	5	50	8	25	3	-	6	50
<i>S. icolados</i>	9	25	7	25	9	12.5	9	3.12	8	25
<i>S. surattense</i>	7	50	8	25	5	50	5	50	5	50
<i>O. compressa</i>	8	25	<log ₂	3.12	6	25	4	-	<log ₂	6.2
<i>N. procumbens</i>	5	50	<log ₂	12.5	<log ₂	6.2	9	3.12	<log ₂	6.2
<i>P. antidotale</i>	<log ₂	6.25	9	12.5	5	50	<log ₂	3.12	5	50
<i>O. esculentum</i>	6	25	3	-	3	-	7	25	9	12.5
<i>S. baryosma</i>	<log ₂	12.5	8	25	<log ₂	12.5	4	-	<log ₂	6.2
Virus control	0	-	0	-	0	-	0	-	0	-

* = IC₅₀ is measured in µg/mL conc. **<log₂ virus quantity mean 0 HA titer

3.2 RT-PCR Assay

RT PCR was performed to quantify SIV H₁N₁ from allantoic fluids collected from *n*-hexane treated eggs. According to the results of this experiment, *n*-hexane extracts of all the selected plants showed strong antiviral activity against SIV H₁N₁, and these results were following the HA test results. The virus was not detected from any sample by RT PCR, and it showed that all *n*-hexane extracts were very effective against SIV H₁N₁. The CT value (30.5755) of virus control confirms that the SIV H₁N₁ titer was high in virus control.

Table 2: RT PCR results of Cholistani Plants

Sr.No	Sample Name	Detectors used	Well	Results
1	<i>H. recurvum</i>	INF.A & PMD-H1	A3	Undetected
2	<i>H. salicornicum</i>	INF.A & PMD-H1	B3	Undetected
3	<i>A. aspera</i>	INF.A & PMD-H1	C3	Undetected
4	<i>S. fruticose</i>	INF.A & PMD-H1	D3	Undetected
5	<i>S. icolados</i>	INF.A & PMD-H1	E3	Undetected
6	<i>S. surattense</i>	INF.A & PMD-H1	F3	Undetected
7	<i>O. compressa</i>	INF.A & PMD-H1	G3	Undetected
8	<i>N. procumbens</i>	INF.A & PMD-H1	H3	Undetected
9	<i>P. antidotale</i>	INF.A & PMD-H1	A4	Undetected
10	<i>O. esculentum</i>	INF.A & PMD-H1	B4	Undetected
11	<i>S. baryosma</i>	INF.A & PMD-H1	C4	Undetected
12	SIV H ₁ N ₁ (control)	INF.A & PMD-H1	F4	30.5755

4. Discussion

Cholistani plants have valuable biological activities, particularly against SIV H₁N₁. Wild plant extracts cover various principal active agents that can exert their antiviral effect either alone or in synergism, thereby providing less time for the virus to mutate and develop resistance against them. These plants can control the growth of SIV H₁N₁ and should be compared with commercially available products i.e., zanamivir, oseltamivir, and Amantadine. According to this research, selected plants were very active for SIV H₁N₁ growth under *ovo* conditions, particularly *n*-hexane and EtOH extracts. In the light of consequences, these plants were grouped into three sets. The first set consists of highly effective extracts of plants that reduced the log₂ from 9 to 5 (HA titer 0 to 32) against SIV H₁N₁. The second set contains those extracts that partially control the virus, and their HA titers were 32 to 256, and the last comprises those extracts of the plants that were minimum effective against H₁N₁. EtOH and *n*-hexane extracts from selected plants belong to group 1 because extracts of all 11 out of 11 plants exhibited significant anti SIV H₁N₁ activity and kept HA titer and CT value of RT-PCR 0 or very low. Ethyl acetate and MtOH extracts belong to group 2 because ethyl acetate extracts of *H. recurvum* & *O. esculentum* and MtOH extracts of *A. aspera* & *O. esculentum* were partially active in controlling growth SIV H₁N₁. Similarly, butanol extracts of *A. aspera*, *S. fruticosa*, *O. compressa*, *S. baryosma* were least effective against H₁N₁ and classified into group 3. The *n*-hexane extracts treated virus was further confirmed through RT PCR. It was noticed that all the extracts were effective in controlling the growth of SIV H₁N₁, and the presence of virus in allantoic fluids was not detected except for virus control.

There are more than a few reports on plant-based antiviral compounds against influenza virus infection. Various antiviral studies have been confirmed on the medicinal plant's species, for example, screening of various plant species including *T. ventricosa*, *C. spicata*, *R. melanophloeos*, *P. viridiflorum* and *C. glabrum* was prominent against influenza virus (Mehrbod *et al.*, 2018). Consistently, leave extracts of *Annona muricata* were also effective against dengue virus type 2 and exposed antiviral activity (Wahab *et al.*, 2018). Padilla *et al.* (2018) also revealed research on the *in vitro* antiviral activity of Brazilian medicinal plants extracts, and these extracts showed antiviral activity against humans (HSV-1), equine (EqHV-1), and swine herpesviruses (SuHV-1). Similarly, MtOH extracts of Cholistani plants have been

tested and found rich in antiviral activities against avian infectious bursal disease (IBD) and infectious bronchitis (IB) viruses (Aslam *et al.*, 2016). In another study, the antiviral potential of Cholistani plants against poultry pathogens was highlighted by Shahzad *et al.* (2020).

Zhang *et al.* (2018) concluded that Shixiangru total flavonoids (STF) could be a good competitor for SIV H1N1 and viral pneumonia medication. The inhibitory action and related molecular mechanism of STF against SIV H₁N₁ were also studied. Similarly, Silva-Mares *et al.* (2018) performed a screening on the north-east Mexican traditional plants (*Salvia texana*, *Persea americana*, *Juglans mollis*, *Salvia ballotae*, *Hamelia patens*, *Ceanothus coeruleus*, *Clematis drummondii*, and *Chrysactinia Mexicana*) to assess the antiviral activities against HSV-1 & 2 on HeLa cells. Sornpet *et al.* (2017) examined *in vitro* antiviral activities of five Asian medicinal plants (*Kaempferia parviflora*, *Gynostemma apenta phyllum*, *Andrographis paniculata*, *Psidium guajava*, and *Curcuma longa*) against AIV. The best inhibitory effect was shown by seed extracts and total alkaloids of *P. harmala* against influenza A virus growth (Moradi *et al.*, 2017). Farid *et al.* (2017) investigated the phytochemical constituents associated with the butanol soluble fraction of *Arum palaestinum* aqueous MtOH extract against avian influenza virus (AIV). Not only plants but also derivatives of camphor have been reported as effective inhibitors in cell culture against AIV (Zarubaev *et al.*, 2015). Along similar lines, the inhibitory action of Chongkukjang (fermented product prepared from soybeans of traditional Korean) against the influenza A virus proved to be very effective (Wei *et al.*, 2015). Assessment of direct antiviral activity against AIV using the extracts of five Asian medicinal plants and Deva-5 herb formulation has also been reported (Oyuntsetseg *et al.*, 2014). Anti SIV H₁N₁ and H₃N₁ activities were observed by the extracts of 50 medicinal plants present in the tropical rainforests of Borneo (Rajasekaran *et al.*, 2013). The significant role of traditional Chinese medicine (TCM) was also reported for anti-influenza activity (He *et al.*, 2011).

Even though our research is introductory in nature as the antiviral characteristics of plants presenting extensive inhibitory effects against the SIV but still need to be interpreted, to the best of our expertise, this is the primary description in scientific literature representing the antiviral potential of plants from the Cholistan desert against SIV H₁N₁. Moreover, the control of viral growth by certain plants is fascinating and should be investigated further. There is a massive gap for clarifying traditional plant utilization into current medication for humans, animals, and domesticated birds like the poultry industry. Another motive to utilize such plant extracts might be the mechanism of activity, e.g., plant-based crops, work synergistically, subsequently giving a shorter time for mutation and producing resistance against drugs. Despite the accessibility of suitable treatments, the interest in antiviral vaccines is increasing.

Furthermore, massive usage of vaccines might become the viable cause of genomic distinction, so screening novel and progressively fresh antiviral compounds from plants or other herbal sources must focus. In addition, deserts with relative natural environments have been additionally found in the Middle East, Africa and Canada, and several other world regions. Highly integrated and collaborative support is required for the antiviral discovery program from natural resources from these areas to combat emerging and existing viral infections.

5. Conclusion

Cholistani plants accomplish a significant role in controlling *in vivo* growth of SIV H₁N₁. Cholistani plants, through their different extracts, control the growth of this virus. Among these, EtOH and *n*-hexane extracts of all the plants were effective against SIV H₁N₁. These results were further confirmed through RT PCR, and results confirmed that both RT PCR and HA test were equally good in determining the

antiviral effect of Cholistan plants. The study strongly supports the idea that Cholistan medicinal plants have strong anti-SIV H₁N₁ effects and offer alternate and cost-effective medication against SIV H₁N₁.

Future Work

The principle active agent(s) from extracts of Cholistan plants will be characterized through LCMS techniques. The extracts of these plants will be tested against other strains of AIV like H₉N₃, H₇N₂, and H₅N₁.

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