Australian Journal of Basic and Applied Sciences, 5(10): 75-92, 2011
ISSN 1991-8178

Screening Of Antiviral Activity Of Some Terrestrial Leaf Plants Against Acyclovir-Resistant Hsv Type-1 In Cell Culture

Shoeib, A.R.S., Zarouk, A.W. and El-Esnawy, N.A.

Water Pollution Department, Environment Research Division, National Research Center, 12311 El-Behoos St., Dokki, Cairo, Egypt.

Abstract: Infections due to Acyclovir (ACV) resistant Herpes Simplex Virus Type 1 (HSV-1) represent an important clinical concern in immunocompromised patients. So, resistance to ACV urged scientists to search for alternatives to the ACV from natural sources. To achieve this aim ACV resistant HSV-1 sample was collected, propagated and adapted onto Vero cell line. Forty two plant extracts (leaf extracts) were tested for the antiviral activity against ACV resistant HSV-1. Contents of plant leafs were extracted using ethyl extraction and some of them by using aqueous extraction due to highly toxicity of their ethyl extracts. Leaf extracts were examined for the cytotoxicity of the leaf contents on Vero cell line to determine the safe doses that will be used for the antiviral assay test against the ACV resistant HSV-1. Chemical composition and mechanism of inhibition were studied for the promising leaf extracts which had antiviral effect on the ACV resistant HSV-1. Also, the protective index was calculated for each promising one. Results showed that the promising leaf plant extracts were *Gossypium barbadense*, *Umbilicus erectus*, *Ficus benghalensis*, and *Aloe vira*, where the maximum inactivation percentage was 99.9, 99.9, 96.6, and 93.3, respectively. *Gossypium barbadens*e and *Umbilicus erectus* leafs had a highly virucidal effect, when they used at a concentration of 20µg or higher. Also, *Ficus benghalensis* leafs extract had a highly virucidal effect and moderate effect on replication at a concentration not less than 80µg, while *Aloe vira* leafs extract had not only virucidal effect but also had effect on replication and adsorption of the virus at a concentration not less than 80µg. The study concluded that some tested leaf plant extracts had excellent percentage for inhibition of the isolated acyclovir resistant HSV-1.

Key words: Acyclovir**,** HSV-1, ACV-resistant HSV-1, Plant's leaf extracts, Anti-HSV activity.

INTRODUCTION

Viral diseases are now considered to be one of the most important and dangerous problems worldwide due to the difficulty of controlling most of them. Because viruses are obligate intracellular parasites, antiviral agents must be capable of selectively inhibiting viral function without damaging the host.

Herpes simplex virus (HSV) is a human virus causes the infections of the skin and mucous membranes and an uncommon cause of more serious infections in other parts of the body. HSV is one of the most difficult viruses to control and has plagued mankind for thousands of years (Roizman *et al*., 2007).

The deoxyguanosine homolog acyclovir (ACV) is the most common drug used to treat Herpes simplex virus type 1 (HSV-1) infections from the past until now (Elion *et al*., 1977). After being taken up into cells, ACV is sequentially converted into ACV monophosphate, ACV diphosphate, and finally its active form ACV triphosphate. The sequential phosphorylations require HSV-encoded Thymidine kinase (TK) and cellular enzymes. ACV triphosphate is more efficiently incorporated into replicating DNA by HSV DNA polymerase than by the cellular DNA polymerase (Vajpayee and Malhotra. 2000). These characteristics of ACV result in its selectivity for virus-infected cells and its extremely low toxicity to uninfected cells. However, if HSV loses its TK function (including an alteration in substrate specificity or the loss of TK activity) or its DNA polymerase has altered substrate affinity, the virus becomes ACV resistant.

While viral TK function is crucial for ACV activity, TK is not essential for HSV-1 to replicate in dividing cells such as human epithelial cells, presumably due to the abundance of nucleotides in these cells. Thus, HSV-1 mutant that is resistant to ACV therapy can still replicate in epithelial cells and cause lesions. In contrast, TK activity is important for virus replication in resting cells or neurons. Though ACV resistant HSV-1 infection rarely has clinical significance in immunocompetent individuals, severe disease can occur in immunocompromised persons such as AIDS patients and bone marrow transplant recipients (Harris *et al.*, 2003).

The emergence of drug resistant strains has underlined the urgency of the discovery of novel anti-HSV-1 drugs. Several hundred plant species that have potential as novel antiviral agents have been studied, with surprisingly little overlap (Manach *et al*., 2004). A wide variety of active phytochemicals, including the

Corresponding author: Shoeib, A.R.S., Water Pollution Department, Environment Research Division, National Research Center, 12311 El- Behoos St., Dokki, Cairo, Egypt.
E-mail: [ashraf_shoeib@yahoo.com].

alkaloids, coumarines, flavonoids, lignans, peptides, polyphenolics, proteins, saponins, steroids, sulphides and terpenoids have been identified. Some volatile essential oils of commonly used culinary herbs, spices and herbal test have also exhibited a high level of antiviral activity (Jassim and Naji. 2003).

However, only few classes of compounds were investigated, most of the pharmacopoeia of compounds in medicinal plants with the antiviral activity is still not known. Several of these phytochemicals have complementary and overlapping mechanisms of action, including antiviral effects by either inhibiting the formation of viral DNA or RNA or inhibiting the activity of viral replication, or inactivate the virus by inhibition of the viral adsorption onto the host cell (Zhang *et al*., 2007).

In similar studies, researchers found that Flavan-4-ol luteoforol, isolated from *Hypericum connatum* (Guttiferae), and used in southern Brazil in the treatment of lesions in the mouth, shows anti-HSV-1 activity, however, it was often related to acute herpetic gingivostomatitis (Fritz *et al*., 2007). The antiviral protein GFAHP was purified from an extract of *Grifola frondosa* fruiting bodies inhibited HSV-1 replication *in vitro* (Gu *et al*., 2007). The antiviral peptide from seeds of *Sorghum bicolor L*. was strongly inhibited the replication of HSV-1, while the presence of the peptide before HSV-1 infections showed moderate inhibition of virus-induced cytopathic effects (CPE) as compared to during or after infections (Filho *et al*., 2008). Also, 1,3,4,6-tetra-O-galloyl-β-D-glucose (1346TOGDG), was isolated from the traditional medicinal plant *Phyllanthus urinaria* and inhibited HSV-1 infection with no toxic effect at the antiviral concentration (Yang *et al*., 2007).

MATERIALS AND METHODS

Virus:

Acyclovir resistant and wild type Herpes simplex virus type 1 (HSV-1) were kindly provided by Dr Kouka Saad El dein, (professor of medical microbiology, Faculty of medicine for girls, Al Azhar university). Virus was provided in high titer as it was propagated and adapted onto African green monkey kidney (Vero) cells. The virus stock was further propagated onto Vero cells, where virus was released from the infected cell cultures that showed CPE by three cycles of freezing at -20°C and thawing at room temperature. Virus stocks were clarified from cell debris by centrifugation at 3000rpm for 15min. Supernatant containing virus harvest was collected and divided into aliquots 1ml each and stored at -80°C. The virus count for each virus stock was calculated using plaque infectivity assay, and the sensitivity of the virus stock to acyclovir was tested.

Cell Line And Virus Quantification:

African Green Monkey kidney cells (Vero) cell line was obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA). The obtained cells were grown, propagated, and preserved in liquid nitrogen for further needs. Vero cells were used for propagation and quantification of HSV-1 stocks for antiviral bioassay. Plaque reduction assay was used for HSV-1 quantification, where a confluent sheet of Vero cells that were grown in plastic tissue culture plates (Greiner, either 12 or 24 well), in growth Dulbecco's Modified Eagle Medium (DMEM, Lonza), supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (penicillin 100U/ml, streptomycin 100µg/ml, amphotericin B 2.5µg/ml, Lonza), were used for quantification of virus for resistant HSV-1 and for antiviral bioassay. Growth medium was aspirated and plates were inoculated with the calculated no of the original virus stock. After thoroughly spreading of the virus over the Vero cell sheet, the inoculated wells were incubated at 37°C for 1hr for virus adsorption, and the inoculums were aspirated. An overlay medium was prepared by melting a suitable volume of 2% agarose and mixed at 47°C with an equal volume of 2x DMEM supplemented with 2% antibiotic-antimycotic mixture and 5% fetal bovine serum. When the overlay medium cooled down to about 42-45°C, appropriate volume was poured on to each well. Plates were left at room temperature for about 20minutes for solidification of the overlay medium. After solidification, plates were incubated at 37°C and observed daily for appearance of cytopathic effect (CPE) plaques. Plates were then fixed using 10-15% formaldehyde for 1hr. The agarose overlay was discarded and the fixed cells were stained using crystal violet staining solution for 5min. After drying of the plates, plaques were counted (George *et al.,* 1996).

Sensitivity Of Herpes Simplex Virus Type1 Isolate To Acyclovir Drug:

Sensitivity of Herpes Simplex Virus type 1 (HSV-1) isolate to acyclovir (ACV [9-(2-hydroxyethoxymethyl) guanine]) was tested using plaque reduction assay on Vero cell line. Briefly, ACV was dissolved in DMSO at a concentration of 10mg/ml, and sterile single packed 12-well tissue culture plates (Greiner) were cultivated with confluent monolayer Vero cells. HSV-1 sample was inoculated (100µl/well) onto Vero confluent monolayer (4wells/sample), and two-fold dilution of ACV from concentration of 5 to 0.6µg were added and run in quadruplicate wells. Inoculates were well spread on the cell sheets and incubated for 1hr at 37°C for virus adsorption. An overlay medium was poured onto each well, and plates were incubated at 37°C and observed daily for formation of plaques. After appearance of plaques, plates were fixed and the agarose over layer was discarded and the fixed cells were stained. Plaques were counted and the half maximal inhibitory concentration

(IC_{50}) was calculated. HSV isolates were considered resistant to ACV when the IC_{50} was > 3µg/ml (Sangdara and Bhattarakosol. 2008).

Preparation Of Plant Leaf Extracts:

Forty two plant leaves from twenty five plant families were collected from the Herbarium of Botany Department, Faculty of Science, Cairo University. Plants were completely dried at temperature not exceeding 40°C, then grinded carefully and finally stored in plastic bags at –80°C till extraction of the plant contents. Plants were extracted by two different methods; aqueous & ethyl extraction.

Aqueous Extraction:

A given plant leaf, that was placed in the deep freeze at –80°C till use, was grinded to fine powder using small amounts of liquid nitrogen in a ceramic mortar and grinding with a ceramic pestle. One gram of the powder was placed into a 20ml Pyrex centrifuge tube containing 10ml of water, and incubated at room temperature for 48h then the tube was placed into a water bath at 40°C for 10min. The extract was centrifuged at 2000rpm for 5min. and all of the particulate plant materials from the grinding was pelleted and a relatively clear liquid (the supernatant) containing the water-soluble compounds of interest was filtered using a 40µm filter disk in the filtration apparatus. The filtrate was lyophilized, and the powdered residue was redissolved in 1ml of DMSO (20%) (Leland, 2006).

Ethyl Extraction:

A given plant leaf was grinded to a fine powder as in aqueous extraction. One gram of the plant powder was placed into a 20ml Pyrex centrifuge tube containing 10ml of ethyl alcohol. The mixture was vortex well, for dissolving of the plant leafs into alcohol, and the tube was left at room temperature tell evaporation of all ethyl alcohol, and exudates of the residual pellet was re-dissolved in 1ml of DMSO (20%) (Leland, 2006).

Cytotoxicity Assay:

This test was done to determine the cell culture safe dose of the dissolved plant leaf extracts. Briefly, sterile, single packed 96-well tissue culture plates (Greiner) were cultivated with confluent monolayer Vero cells and inoculated with a serial dilution (1, 2, 4, 8, 10, 20, 30, 40, 50, 60, 70 and 80µl) for each plant leaf extract. Plates were then incubated at 37°C and CPE was observed daily for 48hr of incubation (Aquino *et al*., 1989).

Antiviral Assay:

Screening of the extracted plants leafs, for antiviral effect to acyclovir resistant HSV-1 isolate, was done by plaque reduction technique using 6-well tissue culture plates (Greiner), and Vero cells. All possible modes of antiviral actions were studied, where extracts were assayed for viral replication inhibition, viral adsorption inhibition to Vero host cells, and virucidal effect.

Inhibition Of Viral Replication Assay:

It was tested by post inoculation of extracts after virus application to cells. A 6-well plate was cultivated with Vero cells (10^5cell/ml) and incubated for 1-2 days at 37ºC for formation of confluent sheet. Virus was diluted to 10^7 PFU/ml then 50µl of the virus stock was applied to the monolayer confluent sheet of cells, and then incubated with shaking for 1hr at 37ºC. Unadsorbed viral particles were removed by washing the cells sheet three successive times with medium without supplements. Plant leafs extracts were applied by different concentrations starting from that one giving high percentage of inhibition. After 1hr of contact time with shaking at 37ºC, 3ml of overlay medium was added to the cell monolayer sheet. The plates were left to solidify and incubated at 37ºC and observed daily until the development of the viral plaques. Cell sheets were fixed in 10% formalin solution for 2hr, and stained with crystal violet staining solution. Virus inoculums inoculated only to cells and treated identically without addition of plant extract and served as control. Viral plaques were counted and the percentage of viral reduction was calculated (Amoros *et al*., 1994).

Inhibition Of Viral Adsorption Assay:

It was tested by subjecting the plant extract to the monolayer sheet of cells for 2hr before virus inoculation. Briefly, 6-well plate was cultivated with Vero cells and seed plant extracts were added to the monolayer sheet cells at concentrations giving high percentage of viral inhibition and incubated for 2hr at 37ºC. Extract was removed by washing the cells three successive times with media without supplements. Virus was diluted to 10^7 PFU/ml then 50µl of the virus stock was applied to the monolayer confluent sheet of cells, and then incubated with shaking for 1hr at 37ºC for virus adsorption. Unadsorbed viral particles were removed by washing the cells sheet three successive times with medium without supplements, and 3ml of overlay medium was added to the cell monolayer sheet. The plates were left to solidify and incubated at 37ºC and observed daily until the development of the viral plaques. Cell sheets were fixed in 10% formalin solution for 2hr, and stained with crystal violet staining solution. Virus inoculums inoculated only to cells and treated identically without addition

of plant extract and served as control. Viral plaques were counted and the percentage of viral reduction was calculated (Zhang *et al*., 1995).

Virucidal Assay:

It was tested by incubation of virus with extract directly before inoculation of virus onto cells. Briefly, 6-well plate was cultivated with Vero cells, and 50µl of virus diluted to 10^7 PFU/ml was added to equal volume (V/V) of plant extract at concentrations giving maximum viral inhibition to give total volume of 100µl. After 1hr of incubation with shaking at 37ºC, the mixture was diluted by 10 fold dilution in a way that still gave suitable count of viral particles. Then 100µl of the mixture were added to the cell monolayer sheet, and incubated with shaking for 1hr at 37ºC, and 3ml of overlay medium was added. The plates were left to solidify and incubated at 37ºC and observed daily until the development of the viral plaques. Cell sheets were fixed in 10% formalin solution for 2hr, and stained with crystal violet staining solution. Virus inoculums inoculated only to cells and treated identically without addition of plant extract and served as control. Viral plaques were counted and the percentage of viral reduction was calculated (Schuhmacher *et al*., 2003).

Qualification Phytochemical Analysis In Plant Leaf Extracts:

Qualification phytochemical analysis to Alkaloids, Flavonoids, Saponins, Steroids, Tannins and Terpenoids for each active plant leaf extract, as anti-HSV-1, was carried out according to the methods described by (Siddiqu *et al*., 2009). Briefly, plant leaves were Grinded to fine powder using small amounts of liquid nitrogen in a ceramic mortar and grinding with a ceramic pestle, and then the plant powder contents were assayed.

Alkaloids

Half gram of the powdered dry plant leaf was boiled with 10ml of dilute hydrochloric acid (alcoholic) in a test tube for 5 minutes. The mixture was cooled and the debris was allowed to settle. The supernatant liquid was filtered into another test tube and 1ml of the filtrate was taken into which three drops of Dragendorffs' reagent (potassium bismuth iodide solution) was added, then the mixture was shaken till appearance of an orange-red spot and a precipitate formation.

Flavonoids:

Ten grams of powdered dry plant leaf was boiled for 2 to 3 minutes in 100ml of water in a water-bath. Three milliliter of the filtrate was added to 3ml of acid -alcohol (Ethanol: Water: concentrated hydrochloric acid in a ratio of 1:1:1), 1cm solid magnesium, and 1ml of t-amyl-alcohol. The mixture was then observed for a rose-orange or violet color change.

Saponins:

Exactly 0.2gm of the powdered dry plant leaf was shaken with water and the mixture was observed for a persistent froth.

Steroids:

One gram of powdered dry plant leaf was extracted for 24 hours in ether, and 1ml of the filtrate was evaporated to dryness and the residue redissolved in several drops of acetic anhydride and then several drops of sulphuric acid were added to solution. The mixture was then observed for a blue, green ring indicated the presence of steroids.

Tannins:

Exactly 0.2gm of the powdered dry plant leaf was boiled in 5ml of water. The mixture was cooled and filtered. A few drops (3 drops) of 5% ferric chloride solution were added to the filtrate and we observed for a blue-black precipitate formation.

Terpenoids:

1gm of powdered dry plant leaf was extracted for 24hr in ether. 1ml of the filtrate was evaporated to dryness and the residue redissolved in several drops of acetic anhydride and then several drops of sulphuric acid were added to solution. The mixture was then observed for a blue, green ring indicated the presence of steroids.

Results:

Sensitivity Of Herpes Simplex Virus Type1 Isolate To Acyclovir Drug:

Results from Sensitivity of Herpes Simplex Virus type1 isolate to Acyclovir drug test showed that even with increase the concentration of ACV HSV-1 gave high number of plaques, which indicated that HSV-1 sample was resistant to ACV drug.

Table 1: Cytotoxicity test of plant extracts on Vero cells.

NO	Plant family	plant name	Dosage											
			1 µg	2 µg	4 µg	8 µg	10 µg	20 µg	30 µg	40 µg	50 µg	60 µg	70 µg	80 µg
1	*Acanthaceae*	*justicia adhatoda*	-	-	-	-	-	-	-	-	-	+	2+	4+
2	*Apocynaceae*	*Vinca rosea*	-	-	-	-	-	-	-	-	-	+	2+	4+
3	*Araliaceae*	*Schefflera arboricola*	-	-	-	-	-	-	-	-	-	+	2+	4+
4	*Asclepiadaceae*	*Hoya carnosa*	-	-	-	-	-	-	-	-	-	+	2+	4+
5	*Asphodelaceae*	*Aloe vera*	-	-	-	-	-	-	-	-	-	-	-	-
6	*Chenopodiaceae*	*Beta vulgaris*	-	-	-	-	-	-	-	-	-	+	3+	4+
7		*Chenopodium ambrsioides*	-	-	-	-	-	-	-	-	-	+	2+	4+
8	*Commelinaceae*	*Tradescantia pallida*	-	-	-	-	-	-	-	-	+	2+	3+	4+
9	*Compositae*	*Helianthus annus*	-	-	-	2+	4+	4+	4+	4+	4+	4+	4+	4+
		*Helianthus annus**	-	-	-	-	+	2+	3+	3+	4+	4+	4+	4+
10	*Convolvulaceae*	*Convolvulus arvensis*	-	-	-	-	-	-	-	-	-	+	2+	4+
11	*Crassulaceae*	*Umbilicus erectus*	-	-	-	+	2+	2+	3+	4+	4+	4+	4+	4+
		*Umbilicus erectus**	-	-	-	-	-	-	-	-	-	-	-	-
12	*Fabaceae*	*Poinciana regia*	-	-	-	-	-	-	-	-	-	+	2+	4+
13	*Lamiaceae*	*Ocimum basilicum*	-	-	-	-	-	-	-	-	-	+	2+	4+
14	*Leguminosae*	*Bauhinia varigata*	-	-	-	-	-	-	-	-	-	+	2+	4+
15		*Cassia Fistula*	-	-	-	-	-	-	-	-	-	+	2+	4+
16		*Lupines termis*	-	-	-	-	2+	4+	4+	4+	4+	4+	4+	4+
		*Lupines termis**	-	-	-	-	-	-	-	-	2+	3+	3+	3+
17		*Phaseolus vulgaris*	-	-	-	-	-	-	-	-	-	+	2+	4+
18	*Leguminosae*	*Trifolium alexandrinum*	-	-	-	-	-	-	-	-	-	+	2+	4+
19		*Vicia faba*	-	-	-	-	-	-	-	-	-	+	2+	4+
20	*Malvaceae*	*Abutilon pictum*	-	-	-	-	-	-	-	-	+	2+	3+	4+
21		*Althea rosea*	-	-	-	-	-	-	-	-	-	+	2+	4+
22		*Hibiscus rosa sinensis*	-	-	-	-	-	-	-	-	-	+	2+	4+
23		*Gossypium barbadense*	-	-	-	-	-	-	-	-	-	-	-	-
24	*Moraceae*	*Ficus benghalensis*	-	-	-	-	-	-	-	-	-	-	-	-
25		*Ficus decora*	-	-	-	-	-	-	-	-	-	+	2+	4+
26		*Ficus nitida*	-	-	-	-	-	-	-	-	-	+	2+	4+
27		*Ficus religiosa*	-	-	-	-	-	-	-	-	-	+	2+	4+
28		*Morus alba*	-	-	-	-	-	-	-	-	-	+	2+	4+
29	*Nyctaginaceae*	*Mirabilis jalapa*	-	-	-	-	-	-	-	-	+	2+	3+	4+
30	*Plantaginaceae*	*Plantago major*	-	-	-	-	-	-	-	-	-	+	2+	4+
31	*Palmae*	*Phoenix dactylifera*	-	-	-	-	2+	4+	4+	4+	4+	4+	4+	4+
		*Phoenix dactylifera**	-	-	-	-	-	-	-	-	+	+	2+	3+
32	*Poaceae*	*Agropyron repens*	-	-	-	-	-	-	-	-	-	+	2+	4+
33	*Punicaceae*	*punica granatum*	-	-	-	-	-	-	-	-	-	+	2+	4+
34	*Solanaceae*	*Datura metel*	-	-	2+	4+	4+	4+	4+	4+	4+	4+	4+	4+
		*Datura metel**	-	-	+	2+	3+	4+	4+	4+	4+	4+	4+	4+
35	*Solanaceae*	*Nicotiana glauca*	-	-	-	-	-	-	-	-	+	2+	3+	4+
36		*Petunia hybrida*	-	-	-	-	-	-	-	-	-	+	2+	4+
37		*Solanum nigrum*	-	-	-	-	-	-	-	-	-	+	2+	4+
38		*Withania somnifera*	-	-	-	+	2+	4+	4+	4+	4+	4+	4+	4+
		*Withania somnifera**	-	-	-	-	-	-	-	-	-	+	2+	3+
39	*Tropaeolaceae*	*Tropeolum majus*	-	-	-	-	-	-	-	-	-	+	2+	4+

40	*Verbenaceae*	*Duranta plumier*	-	-	2+	3+	4+	4+	4+	4+	4+	4+	4+	4+
		*Duranta plumier**	-	-	-	2+	3+	4+	4+	4+	4+	4+	4+	4+
41	*Violaceae*	*Viola tricolor*	-	-	-	-	-	-	-	-	-	+	2+	4+
42	*Vitaceae*	*Vitis vinifera*	-	-	2+	4+	4+	4+	4+	4+	4+	4+	4+	4+
		*Vitis vinifera**	-	-	-	-	+	2+	2+	3+	3+	4+	4+	4+
(*) means the Aqueous extract.														

Cytotoxicity of Plant Leaf Extracts on Vero Cell Line:

Before conducting antiviral activity tests, the cytotoxicity of the extracts on the Vero cells was studied. An antiviral extract should be active against the virus without inducing significant toxicity on the host cell. Therefore, the concentration range in which the extracts will not induce significant toxicity to the host cells was estimated and the cytotoxic concentration was determined for each extract. *Datura metel*, *Duranta plumieri*, *Helianthus annus* and *Vitis vinifera* were highly toxic on Vero cell line in both aqueous and ethyl extraction, therefore these four extracts excluded from anti-HSV-1 bioassays. Aqueous extraction only was used for *Lupines termis, Phoenix dactylifera*, *Umbilicus erectus*, and *Withania somnifera*, due to highly toxicity of the ethyl extraction of these extracts on Vero cell line. Results from Table1 showed that the rate of cell degradation increased with increasing the concentration of some tested toxic extracts, and we found that 20µg and 40µg from each leaf extract were safe on Vero cells in all tested plants, therefore this concentration used in the plaque infectivity count assay against ACV-resistant HSV-1.

Fig. 1: Resistance of Herpes Simplex Virus type1 isolate to Acyclovir drug.

Anti-HSV-1 of Plant Leaf Extracts:

Results showed that Leaf extract of *Gossypium barbadense* and *Umbilicus erectus* had very strong inhibition to HSV-1 resistant to ACV. Leaf extracts of *Agropyron repens, Aloe vira, Bauhinia varigata, Beta vulgaris, Ficus benghalensis, Hoya carnosa, Lupines termis, Mirabilis jalapa, Phaseolus vulgaris, Schefflera arboricola and Tropeolum majus* were had good inhibition range from 50 – >99% and the inhibition increased with increase concentration of extract. Leaf extracts of *Althea rosea, Chenopodium ambrsioides, Hibiscus rosa sinensis, Petunia hybrida, Plantago major*, *Trifolium alexandrinum* and *Vinca rosea* had low inhibition range from 20–50%. Leaf extracts of *Abutilon pictum, Cassia Fistula, Convolvulus arvensis, Ficus decora, Ficus nitida, Ficus religiosa, justicia adhatoda, Morus alba, Nicotiana glauca, Phoenix dactylifera, Poinciana regia, punica granatum, Ocimum basilicum, Solanum nigrum, Tradescantia pallida, Vicia faba, Viola tricolor* and *Withania somnifera* had poor inhibition lower than 20%. Some plant families were represented by more than two plants, since the species belong to the same family are vary in their degree of inhibition. Six species were tested from family *Leguminosae*, where four of them showed good percent of inhibition to ACV-resistant HSV-1, these species were *Bauhinia varigata, Lupines termis, Phaseolus vulgaris and Trifolium alexandrinum* giving , , & % of inhibition, respectively. However two extracts showed poor effect, these species were *Cassia Fistula and Vicia faba* giving & % inhibition, respectively. Four species were tested from family *Malvaceae*, one of them showed complete inhibition to the tested HSV-1, this species was *Gossypium barbadense* giving >99.9% inhibition, two of them showed good inhibition effect, these species were *Althea rosea* and *Hibiscus rosa sinensis* that giving 40 & %, respectively. The remained species had poor effect; this species was *Abutilon pictum* giving 8.57% of inhibition. Five species were tested from family *Moraceae*, one of them showed good inhibition effect to the ACV-resistant HSV-1, this species was *Ficus benghalensis* giving 80% of inhibition. The remained species showed poor effect, these species were *Ficus decora, Ficus nitida, Ficus religiosa and Morus alba* that giving , 6.67, 17.95 & 8.89% of inhibition, respectively. Five species only four of them were tested from family *Solanaceae*, one of them showed moderate effect, this species was *Petunia hybrida* giving % of inhibition and other species showed poor effect, these species were *Nicotiana glauca, Solanum nigrum and Withania somnifera* giving , & % of inhibition, respectively. The antiviral effect of members of families *Leguminosae* and *Malvaceae* were higher than members of families *Solanaceae* and *Moraceae* (Table 2).

Table 2: Inhibitory activity of plant extracts against ACV-resistant HSV-1, using plaque reduction assay.

NO	plant name	Conc. µg	Viral count (PFU/ml) $X10^7$	Initial viral count $X10^7$	% of Inhibition
1	*justicia adhatoda*	20	4.2	4.5	6.67
		40	4.2		
2	*Vinca rosea*	20	3.2	3.9	17.95
		40	3.0		23.08
3	*Schefflera arboricola*	20	3.1	3.9	58.97
		40	3.2		61.54
4	*Hoya carnosa*	20	1.4	4.5	68.89
		40	1.2		73.33
5	*Aloe vira*	20	1.5	3.0	50.00
		40	0.6		80.00
6	*Beta vulgaris*	20	1.5	3.0	50.00
		40	1.4		53.33
7	*Chenopodium ambrsioides*	20	3.3	3.9	15.38
		40	3.1		20.51

Table 2: Continue.

NO	plant name	Conc. µg	Viral count (PFU/ml) $X10^7$	Initial viral count $X10^7$	% of Inhibition
8	*Tradescantia pallid*	20	3.2	3.5	8.57
		40	3.2		
9	*Convolvulus arvensis*	…….	…….	…….	…….
		…….	…….		
10	*Umbilicus erectus*[*]	20	0	3.5	> 99.9
		40	0		
11	*Poinciana regia*	20	3.2	3.5	8.57
		40	3.2		
12	*Ocimum basilicum*	20	4.0	4.5	11.11
		40	4.0		
13	*Bauhinia varigata*	20	0.8	3.0	73.33
		40	0.8		
14	*Cassia Fistula*	20	4.1	4.5	8.89
		40	4.0		11.11
15	*Lupines termis*[*]	20	1.2	3.0	60.00
		40	1.1		63.33
16	*Phaseolus vulgaris*	20	1.5	4.5	66.67
		40	1.5		
17	*Trifolium alexandrinum*	20	2.5	3.9	35.89
		40	2.3		41.03
18	*Vicia faba*	20	2.8	30	6.67
		40	2.6		13.33
19	*Abutilon pictum*	20	3.2	3.5	8.57
		40	3.2		
20	*Althea rosea*	20	2.1	3.0	30.00
		40	1.8		40.00
21	*Hibiscus rosa sinensis*	20	1.8	3.5	48.57
		40	1.8		
22	*Gossypium barbadense*	20	1.0	3.5	71.43
		40	0		> 99.9
23	*Ficus benghalensis*	20	0.8	3.0	73.33
		40	0.6		80.00
24	*Ficus decora*	20	4.1	4.5	8.89
		40	4.0		11.11
25	*Ficus nitida*	20	4.2	4.5	6.67
		40	4.2		
26	*Ficus religiosa*	20	3.4	3.9	12.82
		40	3.2		17.95
27	*Morus alba*	20	4.2	4.5	6.67
		40	4.1		8.89
28	*Mirabilis jalapa*	20	1.5	3.5	57.14
		40	1.4		60.00
29	*Plantago major*	20	2.4	3.9	38.46
		40	2.4		
30	*Phoenix dactylifera*[*]	20	3.2	3.5	8.57
		40	3.1		11.43
31	*Agropyron repens*	20	1.4	4.5	68.89
		40	1.3		71.11

Table 2: Continue.

NO	plant name	Conc. µg	Viral count (PFU/ml) 10^7	Initial viral count X 10^7	% of Inhibition
32	*punica granatum*	20	3.1	3.5	
		40	3.2		
33	*Nicotiana glauca*	20	3.2	3.5	
		40	3.0		
34	*Petunia hybrid*	…….	…….	…….	…….
		…….	…….		
35	*Solanum nigrum*	20	3.4	3.9	
		40	3.4		
36	*Withania somnifera*[*]	20	3.1	3.5	
		40	3.1		
37	*Tropeolum majus*	20	1.8	3.5	
		40	1.6		
38	*Viola tricolor*	20	3.7	3.9	
		40	3.5		
	Tropeolm majus	20	1.8	3.5	
		40	1.6		
	Duranta plumieri	…….	…….	…….	…….
		…….	…….		
	Viola tricolor	20	3.7	3.9	
		40	3.5		
	Vitis vinifera	…….	…….	…….	…….
		…….	…….		

(*) Aqueous extraction only.
(…..) Leaf extract was excluded from anti-HSV-1 bioassays due to toxicity.

Mode Of Inhibition of Plant Leaf Extracts Against Resistant HSV-1 And Their Phytochemical Analysis:

Screening of all plant leaves (Table 2) leads to that there are four promising plants which Subjected to further extensive studies deals with mode of inhibition and their phytochemical analysis.

Aloe Vira:

Leaf extract of *Aloe vira* had highly percentage of inhibition against ACV- resistant HSV-1 (Table 3, Fig. 2&3) where by increasing the concentration of extract the percentage of inhibition was increased.

Table 3: Inhibition activity of *Aloe vira* leaf extract using plaque infectivity count assay against ACV-resistant HSV-1.

plant name	Conc. µg	Viral count (PFU/ml) X 10^7	Initial viral count X 10^7	% of inhibition
Aloe vira	1	3.0	3.0	
	2	3.0	3.0	
	4	3.0	3.0	
	8		3.0	
	10	2.6	3.0	
	20	1.5	3.0	50
	30	1	3.0	66.66
	40	0.6	3.0	80
	50		3.0	
	60	0.4	3.0	

* V.C: Virus control
* C.C: Vero cells control.

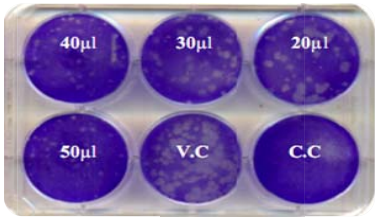


Fig. 2: Plate showed inhibition activity of *Aloe vira* leaf extract using plaque infectivity count assay.

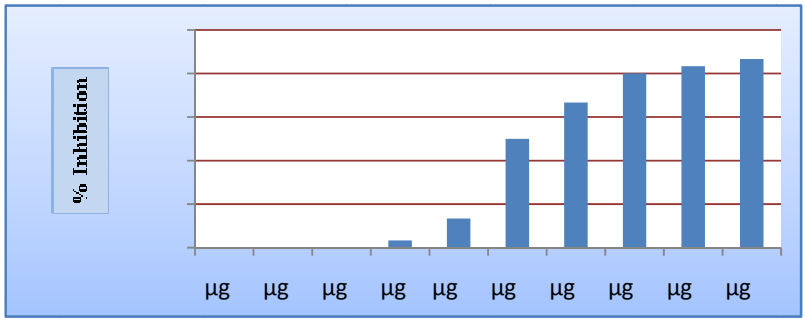


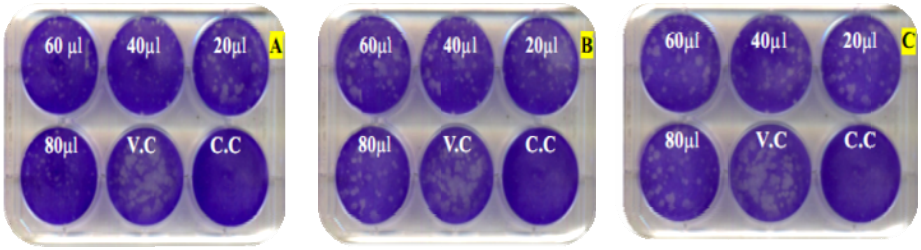
Fig. 3. Histogram showed the inhibition activity of *Aloe vira* leaf extract using plaque infectivity count assay against HSV-1.Where percentage of inhibition increased with increasing the concentration of the *Aloe vira* leaf extract

Mechanism of Action of Aloe Vira Leaf Extract on ACV- Resistant HSV-1:

Results showed that the mode of action of *Aloe vira* leaf extract was virucidal effect (Table 4, Fig. 4&5) also the extract had good inhibition effect on the adsorption and replication of HSV-1.

Table 4: I*n vitro* the inhibition effect of *Aloe vira* leaf extract on ACV- resistant HSV-1.

Conc.	Initial viral count	Viral count (PFU/ml) X 10^7			% inhibition		
		virucidal	Adsorption	Replication	virucidal	Adsorption	Replication
20µg	$3.0x10^7$	1.5	1.9	1.9	50	36.66	36.66
40µg		0.6	1.7	1.7		43.33	43.33
60µg			1.5	1.5		50	50
80µg		0.2	1.3	1.3	93.33	56.66	56.66



* V.C: Virus control, * C.C: Vero cells control.

Fig. 4: The inhibition effect of *Aloe vira* leaf extract using plaque infectivity count assay against ACV- resistant HSV-1. A: virucidal effect, B: effect on adsorption, and C: effect on replication.

Chemical Analysis Of Aloe Vira Leaf Extract:

Chemical analysis of *Aloe vira* leaf extracts showed that it contained alkaloids, flavonoids, saponins, tannins and terpenoids but not contained steroids (Table 5).

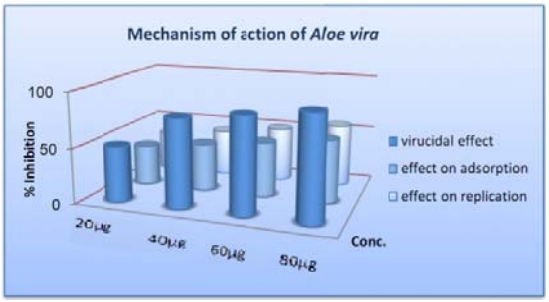


Fig. 5: Histogram showed *in vitro* the inhibition effect of *Aloe vira* leaf extract on ACV- resistant HSV-1.

Ficus Benghalensis:

Leaf extract of *Ficus benghalensis* had highly percentage of inhibition against ACV-resistant HSV-1 (Table 6 and Fig. 6&7). Where by increasing the concentration of extract the inhibition of virus was increased.

Table 5. Chemical analysis of *Aloe vira* leaf extract.

Phytochemical components	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+

Table 6. Inhibition activity of *Ficus benghalensis* leaf extract using plaque infectivity count assay against ACV-resistant HSV-1.

plant name	Conc. µg	Viral count (PFU/ml) X 10^7	Initial viral count X 10^7	% of inhibition
Ficus benghalensis	1	3.0	3.0	.00
	2	3.0	3.0	.00
	4	3.0	3.0	.00
	8	2	3.0	
	10	2.0	3.0	3
	20	0.8	3.0	
	30	0.7	3.0	76.6
	40	0.6	3.0	80.0
	50		3.0	
	60	0.3	3.0	90.0

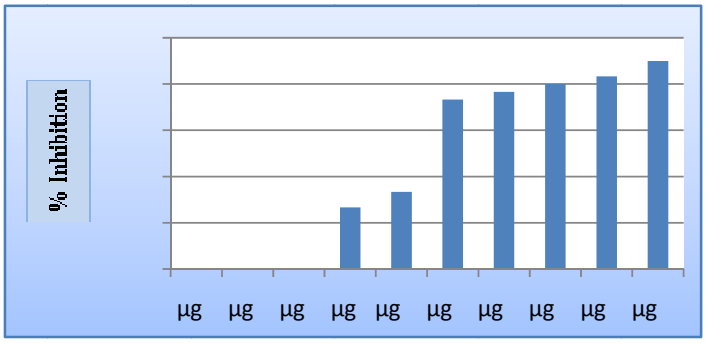
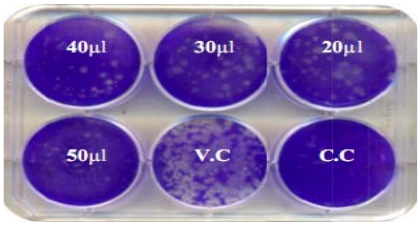


Fig. 6. Histogram showed the inhibition activity of *Ficus benghalensis* leaf extract using plaque infectivity count assay against ACV-resistant HSV-1.Where percentage of inhibition increased with increasing the concentration of the *F. benghalensis* leaf extract.



* V.C: Virus control, * C.C: Vero cells control.

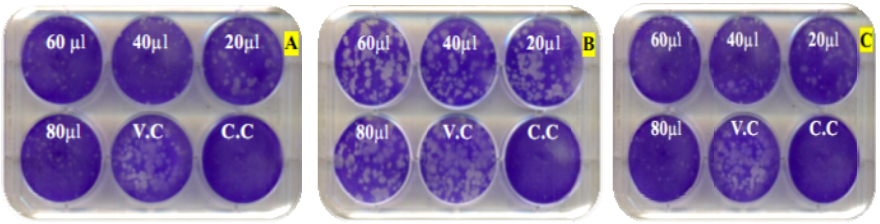
Fig. 7: Inhibition activity of *Ficus benghalensis* leaf extract using plaque infectivity count assay

Mechanism Of Action Of Ficus Benghalensis Leaf Extract On ACV-Resistant HSV-1:

Results showed that the mode of action of *Ficus benghalensis* leaf extract was virucidal effect also the extract had limited effect on the adsorption, and moderate effect on replication of HSV-1, (Table 7).

Table 7. I*n vitro* the inhibition effect of *Ficus benghalensis* leaf extract on ACV- resistant HSV-1.

Conc.	Initial viral count	Viral count (PFU/ml) X 10^7			% inhibition		
		virucidal	Adsorption	Replication	virucidal	Adsorption	Replication
20µg	$3.0x10^7$	0.8	2.8	1.		6.66	
40µg		0.6	2.7		80.00	.00	
60µg		0.3	2.5		.00		
80µg		0.1	2.3		96.66	3.33	.00



* V.C: Virus control, * C.C: Vero cells control.

Fig. 8: I*n vitro* the inhibition effect of *Ficus benghalensis* leaf extract using plaque infectivity count assay against ACV- resistant HSV-1. A: virucidal effect, B: effect on adsorption, and C: effect on replication.

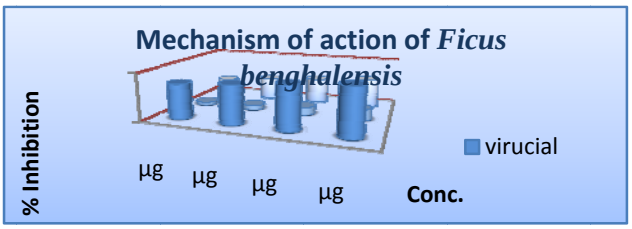


Fig. 9. Histogram showed I*n vitro* the inhibition effect of *Ficus benghalensis* leaf extract on ACV- resistant HSV-1.

Chemical Analysis Of Ficus Benghalensis Leaf Extract:

Chemical analysis of leaf extract of *Ficus benghalensis* showed that it contained flavonoids, steroids and tannin but not contained alkaloid, saponins and terpenoids (Table 8).

Table 8. Chemical analysis of *Ficus benghalensis* leaf extract.

Phytochemical components	Results
Alkaloids	-
Flavonoids	+
Saponins	-
Steroids	+
Tannins	+
Terpenoids	-

Gossypium Barbadense:

Leaf extract *Gossypium barbadense* had highly percentage of inhibition against ACV-resistant HSV-1 starting at 20µg concentration. Where by increasing the concentration of extract the inhibition was increased until it reach to 20µg where the effect became stable at maximum effect (Table 9 and Fig. 10&11).

Table 9: Inhibition activity of *Gossypium barbadense* leaf extract using plaque infectivity count assay against ACV-resistant HSV-1.

plant name	Conc. µg	Viral count (PFU/ml) X 10^7	Initial viral count X 10^7	% of inhibition
Gossypium barbadens	1	3.1	3.5	
	2	3.1	3.5	
	4	3.0	3.5	14.28
	8	1.8	3.5	48.57
	10	1.6	3.5	54.29
	20	0	3.5	> 99.9
	30	0	3.5	> 99.9
	40	0	3.5	> 99.9
	50	0	3.5	> 99.9
	60	0	3.5	> 99.9

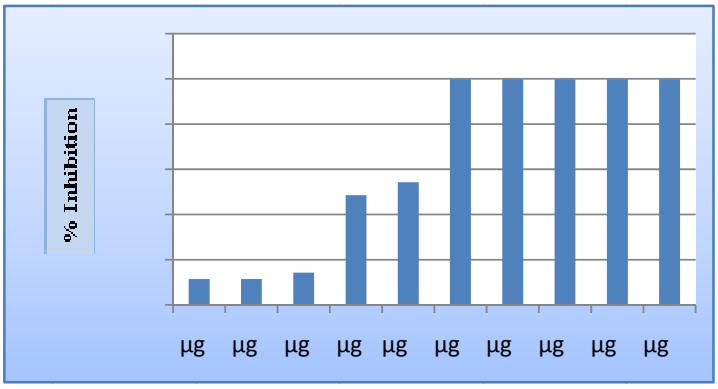
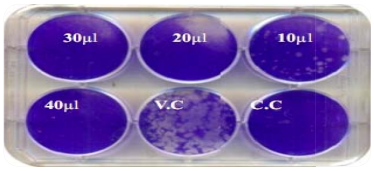


Fig. 10: Histogram showed the inhibition activity of *Gossypium barbadense* leaf extract using plaque infectivity count assay against ACV-resistant HSV-1.

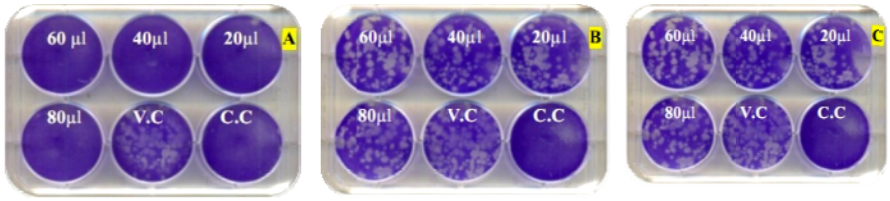


* V.C: Virus control, * C.C: Vero cells control.

Fig. 11: Plate showed the inhibition activity of *Gossypium barbadense* leaf extract using plaque infectivity count assay.

Mechanism Of Action Of Gossypium Barbadense Leaf Extract On Resistant HSV-1:

The mode of action of *Gossypium barbadense* leaf extract was virucidal effect and had limited effect on adsorption or replication of HSV-1.



* V.C: Virus control, * C.C: Vero cells control.

Fig. 12: I*n vitro* the inhibition effect of *Gossypium barbadense* leaf extract using plaque infectivity count assay against ACV- resistant HSV-1. A: virucidal effect, B: effect on adsorption, and C: effect on replication.

Table 10: I*n vitro* the inhibition effect of *Gossypium barbadense* leaf extract on ACV- resistant HSV-1.

Conc.	Initial viral count	Viral count (PFU/ml) X 10^7			% inhibition		
		virucidal	Adsorption	Replication	virucidal	Adsorption	Replication
20µg	$3.0x10^7$	0	2.8	2.8	>99.9	6.66	6.66
40µg		0	2.8	2.8	>99.9	6.66	6.66
60µg		0	2.8	2.8	>99.9	6.66	6.66
80µg		0	2.8	2.8	>99.9	6.66	6.66

Chemical Analysis Of Gossypium Barbadense Leaf Extract:

Chemical analysis of leaf extract of *Gossypium barbadense* contained alkaloid, flavonoids, steroids, tannin and terpenoids but not contained saponins (Table 11).

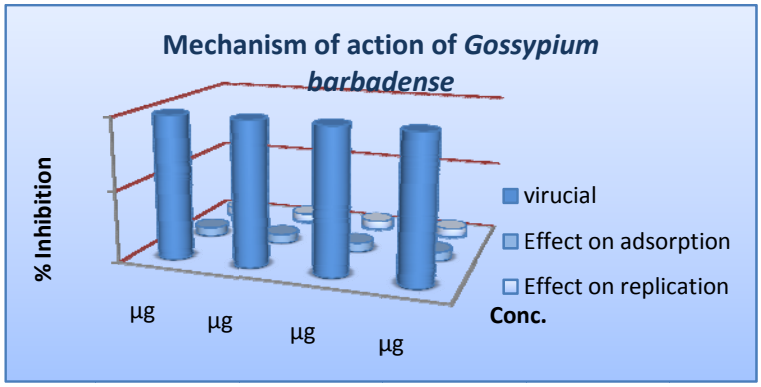


Fig. 13: Histogram showed i*n vitro* the inhibition effect of *Gossypium barbadense* leaf extract on ACV-resistant HSV-1.

Table 11: Chemical analysis of *Gossypium barbadense* leaf extract.

Phytochemical components	Results
Alkaloids	+
Flavonoids	+
Saponins	-
Steroids	+
Tannins	+
Terpenoids	+

Umbilicus Erectus:

Aqueous leaf extract of *Umbilicus erectus* had highly percentage of inhibition against ACV-resistant HSV-1. Where by increasing the concentration of extract the inhibition was increased until it reach to 10µg where the effect became stable at maximum effect (Table 12 and Fig. 14&15).

Table 12: Inhibition activity of *Umbilicus erectus* leaf extract using plaque infectivity count assay against ACV-resistant HSV-1.

plant name	Conc. µg	Viral count (PFU/ml) X 10^7	Initial viral count X 10^7	% of inhibition
Umbilicus erectus	1	1.8	3.5	
	2	1.8	3.5	
	4	1.6	3.5	
	8		3.5	8
	10	0	3.5	> 99.9
	20	0	3.5	> 99.9
	30	0	3.5	> 99.9
	40	0	3.5	> 99.9
	50	0	3.5	> 99.9
	60	0	3.5	> 99.9

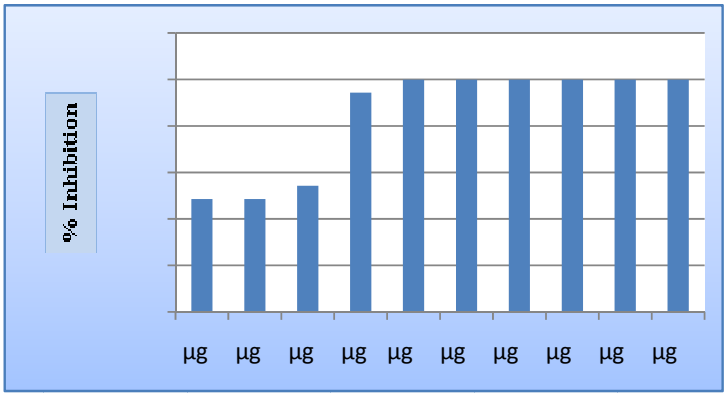
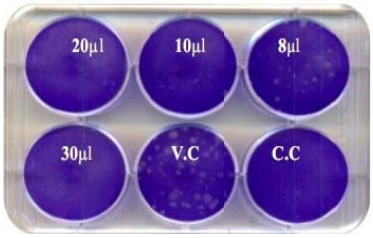


Fig. 14: Histogram showed the inhibition activity of *Umbilicus erectus* leaf extract using plaque infectivity count assay against HSV-1.



* V.C: Virus control, * C.C: Vero cells control.

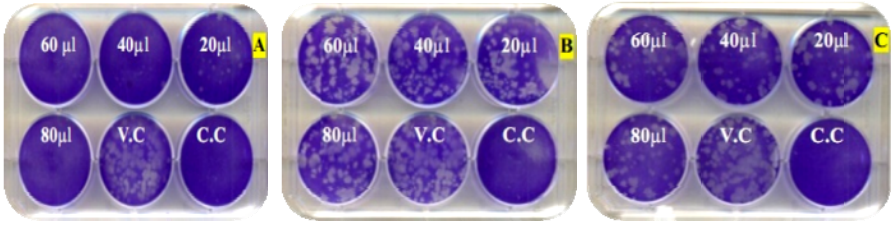
Fig. 15. Inhibition activity of *Umbilicus erectus* leaf extract using plaque infectivity assay.

Mechanism Of Action Of Umbilicus Erectus Leaf Extract On Resistant HSV-1:

The mode of action of *Umbilicus erectus* leaf extract was only virucidal effect and had limited inhibition effect on the adsorption and replication of ACV-HSV-1(Table 13 and Fig. 16 & 17).

Table 13: *In vitro* the inhibition effect of *Umbilicus erectus* leaf extract on ACV- resistant HSV-1.

Conc.	Initial viral count	Viral count (PFU/ml) X 10^7			% inhibition		
		virucidal	Adsorption	Replication	virucidal	Adsorption	Replication
20µg	$3.0x10^7$	0	2.7	2.5	>99.9	10	16.66
40µg		0	2.7	2.5	>99.9	10	16.66
60µg		0	2.7	2.5	>99.9	10	16.66
80µg		0	2.7	2.5	>99.9	10	16.66



* V.C: Virus control, * C.C: Vero cells control.

Fig. 16: *In vitro* the inhibition effect of *Umbilicus erectus* leaf extract using plaque infectivity count assay against ACV- resistant HSV-1. A: virucidal effect, B: effect on adsorption, and C: effect on replication.

Chemical Analysis Of Umbilicus Erectus Leaf Extract:

Chemical analysis of *Umbilicus erectus* leaf extract contained saponins, steroids, tannin and terpenoids but not contained alkaloids and flavonoids (Table 14).

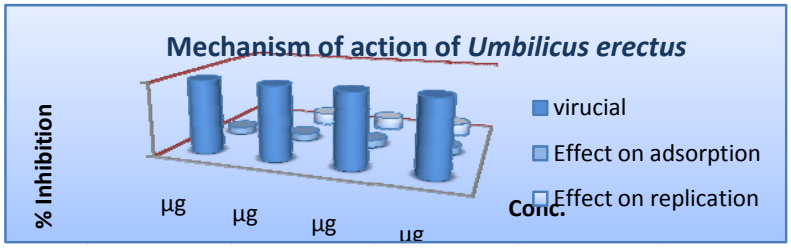


Fig. 17: Histogram showed *in vitro* the inhibition effect of *Umbilicus erectus* leaf extract on ACV- resistant HSV-1.

Table 14: Chemical analysis of *Umbilicus erectus* leaf extract.

Phytochemical components	Results
Alkaloids	-
Flavonoids	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

The Protective Index (Toxicity/Antiviral Activity):

The protective index is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity. Quantitatively, it is the ratio given by the toxic dose divided by the therapeutic dose. A protective index is the toxic dose of a drug for 50% of the population (TD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50}). A high protective index is preferable to a low one.

$$\text{Protective index} = \frac{TD_{50}}{ED_{50}}$$

The protective index is similar to the therapeutic index, but concerns toxicity (TD_{50}) rather than lethality (LD_{50}); thus, the protective index is a smaller ratio. Toxicity can take many forms, as drugs typically have multiple side effects of varying severity, so a specific criterion of toxicity must be specified for the protective index to be meaningful. Ideally a choice is made such that the harm caused by the toxicity just outweighs the benefit of the drug's effect. Thus, the protective index is a more accurate measure of the benefit-to-risk ratio than the therapeutic index, but is less objectively defined.

The protective index of *Aloe vira in vitro* onto cell culture was calculated from results here the (TD50) was 70µg and the (ED50) was 20µg. Therefore, the protective index of *Aloe vira* was 3.5. Similarly, the protective index of the other promising leaf extracts were calculated where it was 4.33 (65µg/15µg) in *Ficus benghalensis*, 7 (70µg/10µg) in *Gossypium barbadense*, and was 16 (80µg/16µg) in *Umbilicus erectus.*

Comparison Between The Effect Of Acyclovir, Aloe Vira, Ficus Benghalensis, Gossypium Barbadense And Umbilicus Erectus On Wild HSV-1:

The effect of Acyclovir (ACV) was compared with the effect of *Aloe vira, Ficus benghalensis, Gossypium barbadense* and *Umbilicus erectus* leaf extracts on the wild HSV-1. Data showed that the antiviral effect of *Aloe vira, Ficus benghalensis, Gossypium barbadense* and *Umbilicus erectus* leaf extracts on the wild and ACV-resistant HSV-1 was the same (Table 15). The antiviral effect of *Gossypium barbadense* and *Umbilicus erectus* leaf extracts more than the effect of ACV but the antiviral effect of *Aloe vira and Ficus benghalensis* leaf extracts were less than the effect of ACV. Combination between ACV and any leaf extract from the four extracts showed complete inhibition to the wild type of HSV-1.

Table 15. Comparison between the antiviral effect of Acyclovir, *Aloe vira, Ficus benghalensis, Gossypium barbadense and Umbilicus erectus* on wild HSV-1.

Conc. (µg) of Acyclovir	%of inhibition	Conc. (µg) of *Aloe vira*	%of inhibition	Conc. (µg) of *Ficus benghalensis*	%of inhibition	Conc. (µg) of *Gossypium barbadens*	%of inhibition	Conc. (µg) of *Umbilicus erectus*	%of inhibition
10	10	10	15.33	10	33.33	10	54.29	10	>99.9
20	15	20	50	20	73.33	20	>99.9	20	>99.9
30	40	30	66.66	30	76.66	30	>99.9	30	>99.9
40	75	40	80	40	80	40	>99.9	40	>99.9
50	>99.9	50	83.33	50	83.32	50	>99.9	50	>99.9

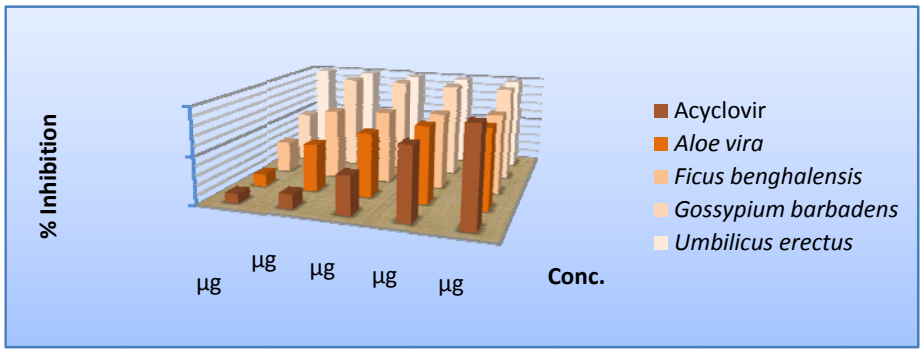


Fig. 18: Histogram showed *in vitro* comparison between the antiviral effect of Acyclovir, *Aloe vira, Ficus benghalensis, Gossypium barbadense and Umbilicus erectus* on wild HSV-1.

Discussion:

Screening efforts have been made to find antiviral agents from natural sources. Plants have long been used as folk remedies, and many are now being collected by ethnobotanists and examined in an attempt to identify

possible sources of antiviral agents. Viruses respond differently to plant extracts and it has been suggested that natural products are preferable to synthetic compounds. Besides a variety of synthetic antiviral drugs with different molecular targets, a large number of phytochemicals, invariability "cocktail of natural products", have been recognized to control infections caused by viruses (Maurice *et al*., 1999). Few classes of compounds investigated most of the pharmacopoeia of compounds in medicinal plants with the antiviral activity is still not known. Several of these phytochemicals have complementary and overlapping mechanisms of action, including antiviral effects by either inhibiting the formation of viral DNA or RNA or inhibiting the activity of viral reproduction (Jassim and Naji 2003).

Different plants were bioassayed against different viruses that can grow on tissue culture like HSV and many were proved to have antiviral activity against HSV-1 and -2 (Benencia *et al*., 1999), other authors reported anti-herpes simplex virus activities of crude water extracts of Thai medicinal plants (Yoosook *et al*., 2000), anti-herpes simplex virus activity of *Geranium sanguineum L.* (Serkedjieva and Ivancheva, 1999), effect of *Annona muricata* and *Petunia nyctaginiflora* extracts on HSV-1 (Padma *et al*., 1998), antiviral activity of hop constituents against HSV-1 and -2. Also, inhibition of HSV- 1 by aqueous extracts from the leaves of *Helichrysum litoreum Guss* (Guarino and Sciarrillo 2003), and antiviral activity of extract from *Echinaceae pallida var.snguinea* against HSV-1(Binns *et al*., 2002). Furthermore, the hot water extract from seeds of *Arachis hypogaea, Pisum sativum*, and soybean blocked herpes simplex and other viruses' infections (Chiang *et al*., 2003 and Yamai *et al*., 2003). Also, crude seeds extract of *Quercus lusitanica,* and Egyptian pea (*Pisum sativum*), and *Nigella sativa* plants have a good inhibitory and antiviral effect on the replication of dengue virus type 2 (Muliawan *et al*., 2006)**,** HCV (Al-Sohaimy *et al*., 2007), and Laryngotrachietis Virus (Zaher *et al*., 2008), respectively.

In the present study forty two plant species belonged to twenty five plant families were collected from the Herbarium of Department of Botany, Faculty of Science, Cairo University. Plant species were collected randomly covering a large number of families and some of them had a history of medical uses. Extraction was made to plants by using ethyl alcohol which is mild in its polarity giving the chance to both polar and non polar compounds to be extracted. Most plant extracts was nontoxic from 20µg to 40µg, so that these two concentrations were selected to anti-HSV-1 bioassays for all plant extracts to compare between them at fixed concentration. For leaf extracts of *Datura metel*, *Duranta plumieri*, *Helianthus annus*, *Lupines termis*, *Phoenix dactylifera*, *Umbilicus erectus*, *Vitis vinifera* and *Withania somnifera* aqueous extraction was done due to highly toxicity of the ethyl extraction of these extracts on Vero cell line. *Datura metel*, *Duranta plumieri*, *Helianthus annus* and *Vitis vinifera* were highly toxic on Vero cell line in aqueous and ethyl extraction. There for these four extracts excluded from anti-HSV-1 bioassays. Ethyl leaf extract of *Aloe vira, Ficus benghalensis and Gossypium barbadense* and aqueous leaf extract of *Umbilicus erectus* had excellent inhibition to resistant HSV-1; therefore we extensively studied them to know the phytochemical composition, mode of inhibition and the protective index to each one.

Aloe vira leaf extract had a good virucidal effect and good inhibition effect on both the adsorption and replication of ACV-resistant HSV-1. *Aloe vira* leaf extract contained alkaloids, flavonoids, saponins, tannins and terpenoids but not contained steroids these findings was agree with (Arunkumar and Muthuselvam. 2009). *Ficus benghalensis* leaf extract had virucidal effect; also the extract had limited effect on the adsorption and moderate effect on replication of HSV-1**.** Chemical analysis of *Ficus benghalensis* showed that it contained flavonoids, phenol, steroids and tannins but not contained alkaloids, carliac, saponins, and terpenoids findings that also agree with Siddiqui *et al*. (2009). *Gossypium barbadense* leaf extract had virucidal effect and had limited effect on adsorption or replication of HSV-1. Leaf extracts were found contained alkaloid, flavonoids, steroids, tannin and terpenoids but not contained saponins. *Umbilicus erectus* leaf extract was had virucidal effect and the extract had limited effect on the adsorption or replication of HSV-1, and the leaf extract contained saponins, steroids, tannin and terpenoids but not contained alkaloids and flavonoids that was agree with previous findings of Kabatende (2005).

Qualitative chemical analysis of the four leaf extracts of *Aloe vira, Ficus benghalensis*, *Gossypium barbadense* and *Umbilicus erectus* showed that all of them contained tannins, and all of them had virucidal effect on ACV-resistant HSV-1. The presence of tannins may interact by direct binding between virus particles either alone or synergistic with certain component in the tested plant extract. Many plant extracts have antiviral activity due to presence of tannins like inhibition of replication of influenza A virus by condensed tannins in *Bergenia ligulata* (Rajbhandari *et al*., 2003), also effect of polyphenolic compounds from medicinal plant *Geranium sanguineum L.* has an effect on HSV replication (Serkedjieva and Ivancheva 1999), and the effect of the presence of tannins in pericarp of *Granatum L*. that was having virucidal effect on genital herpes virus and blocking its adsorption to cells (Zhang *et al*., 1995). In addition, Pharmacological properties of tannins have been investigated based on recent advances in its structural in medicinal plants, as well as its actions as antitumor and antiviral agents. These effects of tannins are attributed to interactions with certain biomolecules in organisms; Tannins are also characterized by their ability to form complexes with proteins (Hatano *et al*., 1999). Thus, this may explain the predominance of the virucidal effect of these four studied plants and/or other plants containing tannins, as they form complex with the capsid (in naked viruses) or the envelope (in enveloped

viruses) preventing the virus from binding with its binding site or cell receptor to cause the infection. Other explanation for the anti-herpes effect is that the components of the tested plants may affect virus adsorption by binding to the receptor site preventing viral attachment, also if any of these components taken inside the cell it might bind with one of the important enzymes needed by the virus and so affect virus replication. Furthermore, the anti-herpes activity of the studied plants is not only due to the tannic compound or extract alone, but also might be as a result of synergetic effect between tannins and another active antiviral compound present in the extract (Hatano *et al*., 1999).

Several chemically defined plant extracts were investigated for their antiviral action on herpes simplex virus (HSV-1, HSV-2)-infected African green monkey kidney cells and human adenocarcinoma cells, using a plaque formation assay. Among them, the monomeric hydrolyzable tannins, oligomeric ellagitannins and condensed tannins, having galloyl groups or hexahydroxydiphenoyl groups, had the most potent anti-HSV activity. Their 50% effective doses (0.03–0.1 μg/ml) were by two-three orders of magnitude lower than their 50% cytotoxic doses (> 10 μg/ml). On the other hand, gallic acid, neutral polysaccharides, chemically modified (N, N-dimethylaminoethyl-, carboxymethyl-, and sulfated-) glucans, sialic acid-rich glycoproteins, and uronic acid-rich pine cone polysaccharide showed little or no activity (Fukuchi *et al*., 1989).

This study concluded that the presence of promising natural antiviral compounds from leaf extract's species, and gave highlight on the importance of natural sources from plant origin in controlling viral diseases. Also, however, other viruses have different structure and different replication cycles, and may their differences in sensitivity to the various plant extracts differs due to the different modes of antiviral action of the active compounds in the plant extracts, the same activity of the studied leaf extracts may be extended to inhibit other members of DNA or may RNA viruses in particular those causing serious diseases to human because the studied HSV-1 representing a member of DNA viruses.

ACKNOWLEDGMENT

We are Thankful to Dr. Koka Saad El-deen, Professor & head of Virology Department, Faculty of medicine for Girls, Al-Azhar University, for her kind gift of the ACV-resistant and wild type HSV-1 isolate. We would also grateful to Dr. El-Sayed Tarek Abd El-Salam, Professor of Virology, Department of Botany, Faculty of Science, Cairo University for his providing with studied plants from the herbarium of the botany department, and his helpful suggestions and discussions. This work was supported by Projects with codes (E8040525) and (E8040802) sponsored by Medical Research Division and Environmental research Division, of National Research Center, respectively. The funding sources had no role in the design of this study.

REFERENCES

Al-Sohaimy, S.A., E.E. Hafez, A.E. Abdelwahab and M.A. El-Saadani, 2007. Anti –HCV Lectin from Egyptian *Pisum sativum*. Australian Journal of Basic and Applied Sciences, 1: 213-219.

Amoros, M., E. Lurton, J. Boustie and L. Girre, 1994. comparison of the anti-herpes simplex virus activities of propolis and 3-methyl-But- 2Enyl cafferate., J. of Natural products., 57: 644-647.

Aquino, R., C.P. Simone, C. Conti and M.L. Stein, 1989. Plant metabolites, structure and *in vitro* antiviral activity of quinovic acid glycosides from *Uncaria tomentosa* and *Guettarda platypoda*, journal of natural products., 52: 679-685.

Arunkumar, S. and M. Muthuselvam, 2009. Analysis of Phytochemical Constituents and Antimicrobial Activities of *Aloe vira*. Against Clinical Pathogens. World Journal of Agricultural Sciences, 5: 572-576.

Benencia, F. and M.C. Courreges, 1999. Antiviral activity of Sandalwood oil against herpes simplex viruses-1 and -2. Phytomedicine. 6: 119-123.

Binns, S.E., J. Hudson, S. Merali and J.T. Arnason, 2002. Antiviral activity of characterized extracts from *Echinacea* Sp. (*Heliantheae: Asteraceae*) against herpes simplex virus type 1. Planta. Med., 68: 780-783.

Chiang, L.C., W. Chiang, M.C. Liu and C.C. Lin, 2003a. *In vitro* antiviral activities of *caesal piniapulcherrima* and its related flavonoides. J.Antimicrob. Chemother., 52: 194-208.

Elion, G.B., P.A. Furman and J.A. Fyfe, 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc Natl Acad Sci USA., 74: 5716-5720.

Filho, I.C., D.G. Cortez and T. Ueda-Nakamura, 2008. Antiviral activity and mode of action of a peptide isolated from Sorghum bicolor. Phytomedicine., 15: 202-208.

Fritz, D., C.R. Venturi and S. Cargnin, 2007. Herpes virus inhibitory substances from *Hypericum connatum Lam*., a plant used in southern Brazil to treat oral lesions.J Ethnopharmaco., 113: 517-520.

Fukuchi, K., K. Sakagami, T. Okuda, T. Hatano, S. Tanuma, K. Kitajima, Y. Inoue, S. Inoue, S. Ichikawa, M. Nonoyama and K. Konno, 1989. Inhibition of herpes simplex virus infection by tannins and related compounds. Antiviral Research., 11: 285-297.

George, G.V., C.J. Hierholzer and W.E. Ades, 1996. Cell culture, Virology Methods Manual. Academic Press Ltd., 1: 1-23.

Gu, C.Q., J.W. Li and F. Chao, 2007. Isolation, identification and function of a novel anti-HSV-1 protein from *Grifola frondosa*. Antiviral Res., 75: 250-257.

Guarino, C. and R. Sciarrillo, 2003. Inhibition of herpes simplex virus type-1 by aqueous extracts from leaves of *Helichrysum litoreum Guss*. Boll. Chim. Farm., 142: 242-243.

Harris, W., P. Collins R.J. Fenton W. Snowden M. Sowa and G. Darby 2003. Phenotypic and genotypic characterization of clinical isolates of herpes simplex virus resistant to aciclovir. J Gen Virol., 84: 1393-1401.

Hatano, T., T. Yoshida and R.W. Hemingway, 1999. Interaction of Flavonoids with peptides and proteins and conformations of dimeric Flavonoids in solution. Plant phenols 2: Chemistry, Biology, Pharmacology, Ecology. New York: Kluwer Academic/ plenum publishers., 509-526.

Jassim, S.A. and M.A. Naji, 2003. Novel antiviral agents: a medicinal plant perspective. J. Appl. Microbiol., 95: 412-427.

Kabatende, J., 2005. Pharmacological evaluation of some central nervous system effects of *Umbilicus erectus*., 37-38.

Leland, J.C., N.S. William, V. Bernhard, A. Kirakosyan and P.B. Kaufman, 2006. Traditional, Analytical, and Preparative Separations of Natural Products. Natural products from plants., 2: 263-317.

Manach, C., A. Scalbert and C. Morand, 2004. Polyphenols: food sources and bioavailability. Am J Clin Nutr., 79: 727-747.

Maurice, M.I., R.D. Angela and O.O. Chris, 1999. New antimicrobials of plant origin. J. Janick (ED.), 457-462.

Muliawan, S.Y., S.K. Lam, D. Shamala, H. Onn and Y. Rohana, 2006. Inhibitory potential of *Quercus lusitanica* extract on dengue virus type-2 Replication. Southeast Asian J. Trop Med Public Health., 37: 132-135.

Padma, P., N.P. Pramod, S.P. Thyagarajan and R.L. Khosa, 1998. Effect of the extract of *Annona muricata* and *Petunia nyctaginiflora* on HSV-1. J.Ethanopharmacol., 61: 81-83.

Rajbhandari, M., U. Wegner, M. Julich, T. Schopke and R. Mentel, 2001. Screening of *Nepalese* medicinal plants for antiviral activity. J. of Ethnopharmacol., 74: 251-255.

Roizman, B., D.M. Knipe and R.J. Whitley, 2007. Herpes Simplex Viruses. Fields Virology. Lippincott Williams & Wilkins., 5: 2503- 2601.

Sangdara, A. and Parvapan Bhattarakosol, B., 2008. Acyclovir Susceptibility of Herpes Simplex Virus Isolates at King Chulalongkorn Memorial Hospital, Bangkok. J Med Assoc Thai., 91: 908-912.

Schuhmacher, A., J. Reichling, and P. Schnitzler, 2003. Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 *in vitro*. Phytomedicine. 6: 504-510.

Serkedjieva, J. and S. Ivancheva, 1999. Antiherpes virus activity of extracts from the medicinal plant *Geranium sanguineum L.*, J. Ethnopharmacol., 64: 59-68.

Siddiqui, S., A. Verma, A.A. Rather, F. Jabeen and M.K. Meghvansi, 2009. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Advances in Biological Research., 3: 188-195.

Vajpayee, M. and N. Malhotra, 2000. Antiviral drugs against Herpes infections. Indian Journal of Pharmacology., 32: 330-338.

Yamai, M., K. Tsumura, M. Kimura, S. Fukuda, T. Murakami and Y. Kimura, 2003. Antiviral activity of a hot water extract of black soybean against a human respiratory illness virus. Bioscience, Biotechnology and Biochemistry., 67: 1071-1079.

Yang, C.M., H.Y. Cheng and T.C. Lin, 2007. The in vitro activity of geraniin and 1, 3, 4, 6-tetra-O-galloyl-β-D-glucose isolated from *Phyllanthus urinaria* against herpes simplex virus type 1 and type 2 infection. J Ethnop- harmaco., 110: 555-558.

Yoosook, C., N. Bunyapraphatsara, Y. Boonyakiat and C. Kantasuk, 2000. Anti-herpes simplex virus activities of crude water extracts of *Thai* medicinal plants. Phytomedicine., 6: 411-419.

Zaher, K.S., W.M. Ahmed and S.N. Zerizer, 2008. Observations on the Biological Effects of Black Cumin Seed (*Nigella sativa*) and Green Tea (*Camellia sinensis*). Global Veterinaria., 2: 198-204.

Zhang, J., B. Zhan, X. Yao, Y. Gao and J. Shong, 1995. Antiviral activity of tannin from the pericarp of Punica granatum L. against genital Herpes virus in vitro. Zhongguo Zhong Yao Za Zhi., 20: 556-576.

Zhang, Y., P.P. But and V.E. Ooi, 2007. Chemical properties, mode of action, and *in vivo* anti-herpes activities of a lignin-carbohydrate complex from *Prunella vulgaris*. Antiviral Res., 75: 242-249.