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THE ANTIVIRAL AND ANTIOXIDANT ACTIVITY OF SOME MEDICINAL PLANTS

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ABSTRACTS

Back ground: Viral infections still remain a serious worldwide problem. The use of synthetic antiviral drugs is often unsatisfactory, as they may be accompanied with a rise of mutant viruses and side or toxic effects besides their high costs.[1] Plants may serve as promising sources of novel antiviral agents.[2] **Aim of the work**: is to test extracts of ten medicinal plants (seven essential oils and three ethanolic extracts) for their antiviral activity against HSV-1 & HAV as well as their antioxidant activity. **Materials & methods:** seven essential oils and three ethanolic extracts were screened for their inhibitory effect against HSV-1 & HAV *in vitro* on Vero cells using a plaque reduction assay. The antioxidant activity was also determined using Thiobarbutiric acid reactive substance. Plant extracts were assayed for their cytotoxicity prior to testing in antiviral studies to determine the maximum noncytotoxic dose. **Results**: HSV-1 was more sensitive towards plant extracts than HAV. All tested extracts had no anti-adsorption or anti-replication effects with the exception of ethanolic extract of *Glycyrrhiza glabra. Lemongrass, mint* and *Tea tree* oil showed high antiviral activity against HSV-1. *Thyme* and *Eucalyptus* showed moderate antiviral activity while *Geranium* and *Plantago* showed low antiviral activity. The plant extracts had antioxidant activity in descending order. **Conclusions**: Essential oils or ethanolic extracts with antiviral activity may be potentially useful cheaper alternative

antiviral agents and pose little threat to induce resistance. They can also be used as food preservatives to control hepatitis outbreaks.

KEYWORDS: HSV-1 & H AV, Plaque Assay, Medicinal plants Antiviral & Antioxidant activity.

BACKGROUND

Viral infections still remain a serious worldwide problem. There is no known specific treatment for viral diseases and limited therapeutic efficacy of most drugs has led to a dependence on preventive measures using vaccines. Nucleoside analogues and other synthetic compounds have traditionally been the primary sources for antiviral agents. The use of antiviral synthetic drugs is often unsatisfactory and limited. Mutant viruses resistant to the existing antiviral agents arise upon treatment or these agents may cause side or toxic effects besides their high costs.[3,4,1] Higher plants may serve as promising sources of novel antiviral agents.[5,6,2] Essential oils are lipophilic multi-component systems with a characteristic pattern of mainly monoterpenes, sesquiterpines and phenylpropanoids.[7] The specific combination of these compounds determines their different biological activities (antimicrobial, antioxidant and cytotoxic).HSV-1 is a member of the family *herpesviridae* subfamilies *Alpha-herpesvirnae,* have large enveloped DNA viruses with an icosahedral capsid and is a highly prevalent pathogen among children and adults, causing primary infections which present clinically as herpes labialis or as primary herpetic gingivostomatitis and is able to establish a latent infection in the nervous system that can be reactivated quite frequently.[8] Human hepatitis A, a widespread infectious disease that is hyperendemic in vast areas of the world, results from infection of the liver by hepatitis A virus (HAV). HAV was first identified in 1973, as a single stranded RNA nonenveloped virus belonging to the *Picornaviridae* family. In fully symptomatic cases the patient presents with jaundice, fever, anorexia, nausea, vomiting, headache and fatigue. In non-complicated cases recovery occurs between 4 and 6 weeks, but fulminating cases may be fatal.[9]

Free radicals are highly reactive and toxic molecules generated in cells under normal metabolic activities. However, in response to a variety of factors including tobacco smoke, pollutants, ionising radiations, alcohol, synthetic pesticides, and solvents, their production increases.[10] Free radicals can cause oxidative damage to proteins, lipids, enzymes, and DNA, and they have been also linked to pathogenesis of oxidative diseases.[11] Living cells possess an excellent scavenging mechanism to avoid excess Free radicals -induced cellular

injury; however, with ageing and under influence of external stresses, these mechanisms become inefficient, and dietary supplementation of synthetic antioxidants is required. In recent years, there has been an increased interest in the use of natural substances as food preservatives and antioxidants.[12] In this context, aromatic plants, particularly their essential oils, are being evaluated for antioxidant activity. It is thus pertinent to evaluate the natural antioxidant activity of essential oils, since they find extensive use in the food and beverage industry.[13,14]

Although natural products have been used since ancient times, only in recent decades has there been growing research into alternative therapies and the therapeutic use of natural products, especially those derived from plants.[15]

AIM OF THE WORK

Is to test extracts of ten medicinal plants (seven essential oils and three ethanolic extracts) for their antiviral activity against HSV-1 & HAV as well as their antioxidant activity.

MATERIALS AND METHODS: PLANTS USED

Ten species of plants belonging to different families were collected from Orman Botanical Garden, Giza, Egypt; Sekem Company, Cairo, Egypt and Medicinal Plant Research Department, Ministry of Agriculture, Giza, Egypt.

Table 1: Medicinal plants used in the present study.

Botanical name	Common name	Family	Extract	Part used
Cymbopogon citratus	Lemongrass	*Poaceae*	Essential oil	leaves
Mentha piperita	Peppermint	*Lamiaceae*	Essential oil	leaves
Melaleuca alternifolia	Tea tree	*Myrtaceae*	Essential oil	leaves
Eucalyptus globulus	Eucalyptus	*Myrtaceae*	Essential oil	leaves
Ocimum basilicum	Basil	*Lamiaceae*	Essential oil	leaves
Pelargonium graveolens	Geranium	*Geraneacee*	Essential oil	leaves
Thymus vulgaris	Thyme	*Lamiaceae*	Essential oil	leaves
Glyzirrhiz glabra	Liquorice	*Fabeacea*	Ethanolic	Roots
Plantago major	Plantago	*Plantaginae*	Ethanolic	leaves
Zizyphus spina christi	Zizyphus	*Rhamnacee*	Ethanolic	leaves

Tissue culture cells

Vero cells (African green monkey cells) were grown in monolayer culture with Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS), 100 ug/ml penicillin and 100 ug/ml streptomycin. Cells were plated out onto 96-well, 6-well and 12- well culture plates for cytotoxicity, viruses titration and antiviral assays, respectively ,and incubated at 37 ºC and 5% CO2.

Viruses

Herpes simplex virus type-1 and Hepatitis A virus at concentrations ranging from 400-500 PFU/ml were used for all experiments. Herpes simplex and Hepatitis A virus were provided by virology lab, Microbiology Department, Faculty of Medicine for Girls, Al -Azhar University, Cairo, Egypt).

Plant extraction

A-Ethanolic extract

The plant materials (*Plantago major, Zizphus spina christi* and *Glyzirrhiz glabra*) were air-dried and ground to a fine powder. Extraction was performed by soaking samples (50 g dry weight) in 70% ethanol (500 ml) for 24 h at 20°C. After filtration, the residues were washed twice with 70% ethanol, followed by drying using a rotary evaporator at 35°C [16].

B-Essential oils extraction

One hundred grams of the fresh plant materials *(Cymbopogon citratus, Mentha piperita, Melaleuca alternifolia, Eucalyptus globules, Ocimum basilicum, Pelargonium graveolens* and *Thymus vulgaris)* were cut into small pieces and submitted for 4h to hydro-distillation. The oils obtained were dried with anhydrous sodium sulphate, and stored in dark vials at 4ºC.[16]

Cytotoxicity assay for plant extracts

Plant extracts (seven essential oils and three ethanolic extracts) were tested for cell toxicity prior to testing in antiviral studies. Cytotoxicity measurements were based on two parameters: (1) Alteration of normal cell morphology (2) Viability of the cells present in the culture.[17]

Titration of viruses (HSV -1 and HAV**) by plaque assay**

Plaque reduction assays are considered the standard technique for assessing the *in vitro* antiviral activity of extracts. Normal replication of a virus in a host cell monolayer results in the formation of plaques or areas of damage to the monolayer. Plaque reduction assays are based on the principle that antiviral agents present in the medium will inhibit the formation of viral plaques. The degree to which plaque formation is inhibited is taken as an indication of the antiviral activity of the compound. **Steps for plaque assay** the[18] technique was followed.

Mechanisms of virus inhibition

Virus inhibition mechanism for all extracts was studied in three categories:

a. Virucidal activity; tested by subjecting virus to extract directly.[19]

b. Prevention of viral adsorption (anti-adsorption); tested by subjecting cells to extract for 1hour before virus inoculation.[20]

c. Prevention of viral replication (anti-replication); tested by post inoculation of extract after 1 h of virus application to cells.[21]

a. Virucidal assay for plant extracts against Herpes simplex virus and Hepatitis A virus: The essential oils of *(Cymbopogon citratus, Mentha piperita, Melaleuca alternifolia, Eucalyptus globules Ocimum basilicum, Pelargonium graveolens* and *Thymus vulgaris)* and the ethanolic extracts of (*Plantago major*, *Zizyphus spina christi* and *Glyzirrhiz glabra*) were tested for their virucidal action against Herpes simplex virus and Hepatitis A virus. Tubes containing 400-500 plaque forming units (PFU) of Herpes simplex virus 1 (HSV-1) or Hepatitis A virus (HAV) were incubated for one hour at 37 °C, in DMEM containing various concentrations of the plant's essential oils or ethanolic extracts. Since the initial dilution of the extract was done in ethanol, an additional tube containing the virus and the appropriate amount of ethanol was used as control and another tube free of both virus and plant extracts as cell control. At the end of the incubation period, 200 *ul* of viral sample was assayed for remaining infectivity on Vero cells by plaque assay.

b. Prevention of viral adsorption (anti-adsorption)[20]

Vero cell monolayers were pretreated with each extract for 3 hours at 37°C prior to virus infection. After washing with phosphate buffered saline (PBS), the cells were infected with HSV-1 or HAV at concentration 400-500 PFU/ml for about 1 hour at 37°C.The unadsorbed viruses were removed and the infective virus was titrated by plaque assay as mentioned before.

c. Prevention of viral replication (anti-replication)[21]

This procedure was done by post inoculation of extract after virus application to cells. Vero cell monolayers were pretreated with HSV-1or HAV with concentration 400-500 PFU/ml for one hour at 37°C prior to extract addition. After washing with PBS, the extracts were added for 1 hour at 37°C.The unabsorbed viruses were removed and the infective virus was titrated by plaque assay as mentioned before.

Antioxidant Assay

Seven essential oils *(Cymbopogon citratus, Mentha piperita, Melaleuca alternifolia, Eucalyptus globules, Ocimum basilicum, Pelargonium graveolens* and *Thymus vulgaris* were evaluated for their antioxidant properties at final concentration of 25 to 100 ppm, using thiobarbituric acid reactive substance (TBARS) method.[22] Absorbance measurements were made using a photometer 5010 (B.M. Germany) set at 546 nm. The antioxidant Index (AI) was expressed as a value relative to the control, calculated using the formula described by[23] AI =(1 – T/C) X 100**.** Where C is the absorbance value of the fully oxidized control and T is the absorbance value of the test samples. This formula was used to demonstrate the comparative protective antioxidative properties of the seven essential oils in the assay.

Statistical analysis

All experiments were performed in triplicate, and three independent experiments were conducted. Data were presented as mean ± SD.

RESULTS: CYTOTOXICITY ASSAY

High cytotoxic effect was recorded for *Glycyrrhiza glabra* ethanolic extract, *Thymus vulgaris* and *Pelargonium graveolens* essential oils where the maximum-nontoxic doses were 50, 100 and 100 ppm respectively. Moderate cytotoxic effect was recorded for *Cymbopogon citratus*, *Ocimum basilicum* , *Ziyzphus spina christi, Mentha piperita* and *Plantago major* where their maximum-non toxic doses were 150, 150, 150, 200 and 200, ppm respectively. Low cytotoxic effect was recorded for *Eucalyptus globule* and *Melaleuca alternifolia* where their maximum-non toxic doses were 300 and 600 ppm respectively. The non-toxic concentration of alcohol on Vero cells was 1%.

Antiviral activity of plant extracts

The antiviral activity of plant extracts was estimated by plaque reduction assay by adding different concentrations of the extract to the virus suspension containing 400-500 plaque forming units (pfu mL−1) for one hour before mixing with the host cells and the results were recorded after 72 hours. The results showed that **HSV-1 was more sensitive towards plant extracts than HAV**. Plaque formation was significantly reduced when HSV-1treated with *Glycyrrhiza glabra,Thymus vulgaris, Cymbopogon citratus, Mentha, piperita, Eucalyptus globules and Melaleuca alternifoli,* while HAV was only affected by *Glycyrrhiza glabra, Thymus vulgaris, Cymbopogon citratus* and *Mentha piperita* extracts.

Results of virucidal activity: HSV1 showed that the rate of plaque inhibition was 90, 84, 90, 40, 30, 60, 70, 88, 35 and 40% when the virus treated by the maximum non-toxic dose of *Cymbopogon citratus, Mentha piperita, Melaleuca alternifolia, Pelargonium graveolens, Ocimum basilicum, Eucalyptus globules, Thymus vulgaris, Glyzirrhiz glabra, Zizyphus spina Christi* and *Plantago major* extracts respectively. While the rate of plaque inhibition of HAV was 60, 50, 30, 20, 10, 20, 55, 70, 15 and 20% when the virus was treated by the maximum non-toxic dose of *Cymbopogon citratus, Mentha piperita, Melaleuca alternifolia, Pelargonium graveolens, Ocimum basilicum Eucalyptus globules, Thymus vulgaris Glyzirrhiz glabra, Zizyphus spina Christi* and *Plantago major* extracts respectively (Table 2; Fig. 1&2).

Mechanisms of the inhibitory effect of the plants extracts

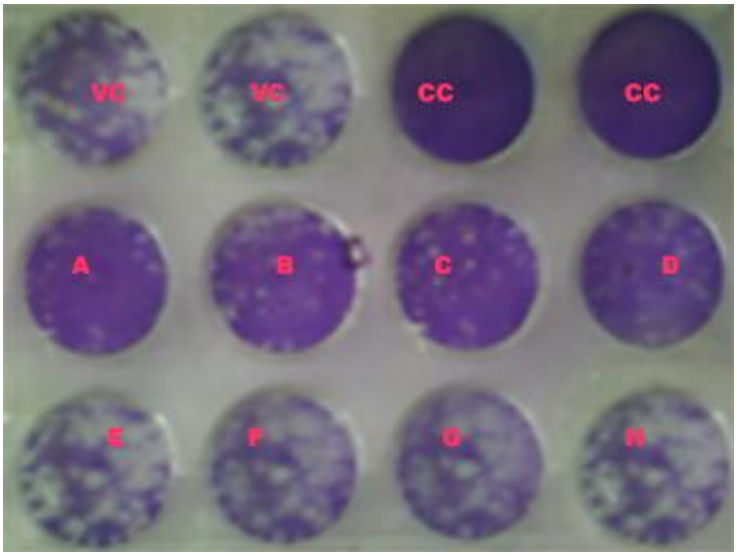
Plants extracts were added at different stages during the viral infection cycle (pretreatment of cells by extract prior to infection to test anti-adsorption effect and post treatment of cells by extract after infection by virus to test the anti-replication). No inhibitory effect was observed when the essential oils were added to the cells prior to infection with each of the two viruses or after the adsorption period. All extracts have no anti-replication or anti-adsorption with the exception of **ethanolic extract** of ***Glycyrrhiza glabra*** which caused plaque inhibition during adsorption stage and replication stage by 70 and 60% respectively for HSV-1 and by 60 and 55% for HAV(Table 2).

Table 2: Antiviral activity of the studied plant extracts against HSV-1and HAV.

Plant Extract	% of plaque reduction at the maximum non-toxic dose of the extract					
	HSV-1			HAV		
	Virucidal	Anti-adsorption	Anti-replication	Virucidal	Anti-adsorption	Anti-replication
C. citrates	**90***	30	30	**60**	20	15
M. piperita	**84**	25	20	**50**	20	20
M. alternifolia	**90**	15	15	30	10	10
P. graveolens	40	30	25	20	10	20
O. basilicum	30	15	20	10	15	10
E. globules	**60**	20	25	20	10	20
T. vulgaris	**70**	15	20	**55**	20	20
G. glabra	**88**	**70***	**60***	**70**	**60***	**55***
Z. spina christi	35	30	35	20	10	10
P. major	40	35	20	20	35	20

***** Only ethanolic extract of ***Glycyrrhiza glabra*** caused plaque inhibition during adsorption and replication stages for HSV-1 and HAV.

**HSV-1 was more sensitive towards plant extracts than HAV.



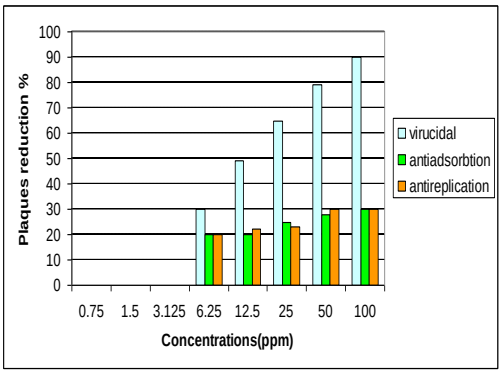


Fig.(1 a and b): Plaque reduction assay and antiviral activity of *Lemongrass* essential oil against HSV-1.

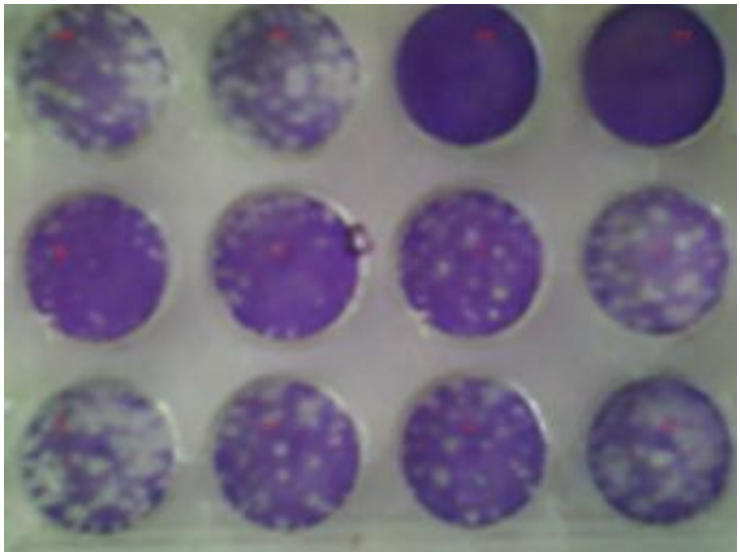
VC: virus control (400-500) PFU of HSV-1 only

CC: Vero cell only and 1% ethanol

A –H: indicating the concentrations of the extract diluted in MEM and ethanol

A: 200 ppm **B**: 100ppm **C**: 50ppm **D**: 25ppm

E: 12.5 ppm **F**: 6.125ppm **G**: 3. 0 ppm **H**: 1. 5ppm



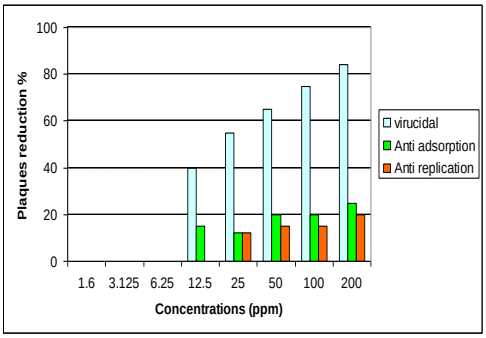


Fig. (2 a and b): Plaque reduction assay and antiviral activity of *Peppermint* essential oil against HSV-1.

VC: virus control (400-500) PFU of HSV-1 only

CC: vero cell only and 1% ethanol

A –H: indicating the concentrations of the extract diluted in MEM and ethanol.

A: 200ppm **B**: 100ppm **C**: 50ppm **D**: 25ppm

E: 12.5ppm **F**: 6.125ppm **G**: 3. 0 ppm **H**: 1. 5ppm

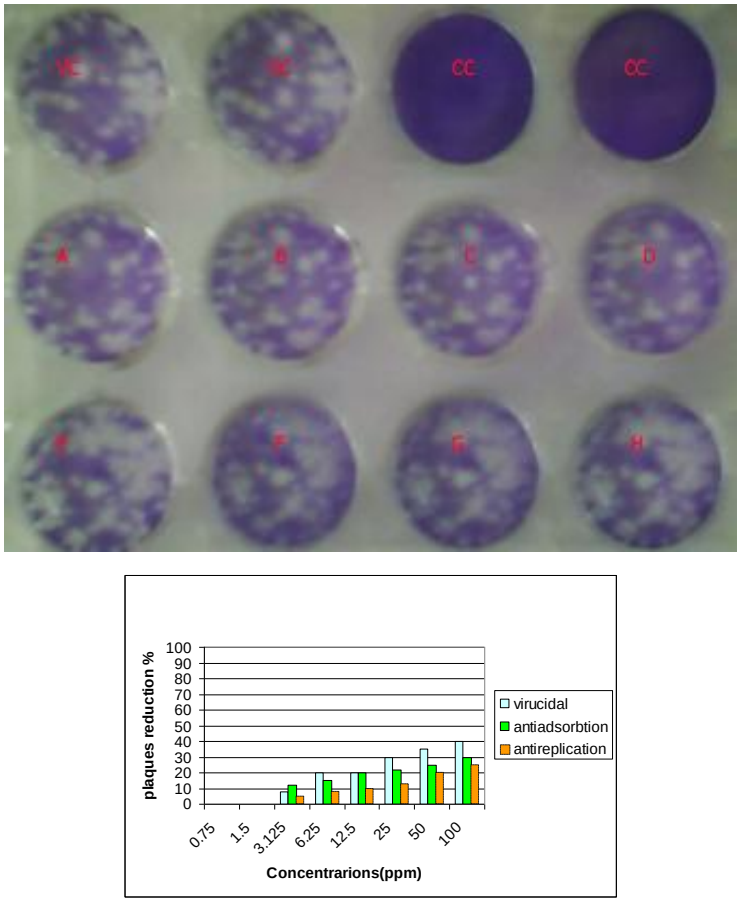


Fig. (3 a and b): Antiviral activity of *geranium* essential oil against HSV-1 using Plaque reduction assay.

VC: Virus control (400-500) PFU of HSV-1 only

CC: Vero cell only and 1% ethanol

A –H: Indicating the concentrations of the extract diluted in MEM and ethanol.

A: 100 ppm **B**: 50 ppm **C**: 25 ppm **D**: 12.5 ppm

E: 6.25 ppm **F**: 3.125 ppm **G**: 1. 5 ppm **H**: 0.7 5 ppm.

Antioxidant Activity

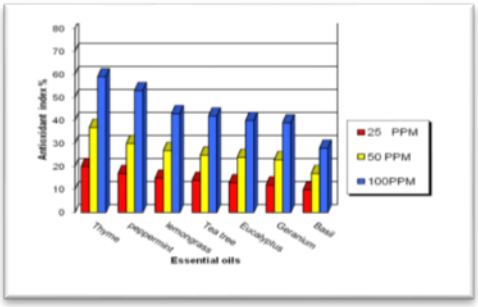
The antioxidant activity of essential oils was assessed by Thiobarbutiric Acid Reactive Substrate (TBARS).All values were based on the antioxidant index, where the control is completely peroxidized and each oil providing a degree of improvement as indicated by % protection. The results showed the antioxidant activities of the seven essential oils. At concentration of 25 PPM the results were as follows in descending order. Thyme oil had the highest antioxidant Index(20%), followed by *Peppermint* oil (17%),*Lemongrass* oil (15%), *Tea* tree oil (14%) *Eucalyptus* oil (13%), *Geranium* oil (12%) and *Basil* oil (9.8%).

While at concentration of 50 PPM the results were as follow 37, 30, 27, 25, 24, 23 and 17% for the essential oils of *Thyme*, *Peppermint*, *Lemongrass*, *Tae* tree oil, *Eucalyptus*, *Geranium* and *Basil* respectively.

At the highest concentration (100 PPM), the results of antioxidant Index for the essential oils of *Thyme*, *Peppermint*, *Lemongrass*, *Tae* tree, *Eucalyptus*, *Geranium* and *Basil* were as follow 59,53, 43 ,42, 40, 39, and 28% respectively (**Table 3 & Fig.4)**.

Table (3) and Fig. (4): Antioxidant activity of some essential oils using TBARS.

Essential oils	Antioxidant Index(%)		
	25 PPM	50 PPM	100 PPM
T. vulgaris (Thyme)	**20 ±0.98***	**37 ±1.99***	**59 ±4.41***
M. pipertia (peppermint)	**17 ±0.69***	**30 ±2.01***	**53 ±2.11***
C citrates(lemongrass)	**15 ±0.88 ***	**27 ±0.88***	**43 ±3.51***
M. altrenifolia(Tea tree)	**14 ±0.81***	**25 ±1.41***	**42±3.51***
E.globules (Eucalyptus)	**13 ±0.91***	**24 ±1.61***	**40 ±3.51***
P.graveolens (Geranium)	**12 ±0.71 ***	**23 ±1.61***	**39 ±2.61***
O.basilicum (Basil)	**9.8** ±0.55*	**17±0.51***	**28 ±0.99***



*The mean ±SD was calculated in triple independent experiments

DISCUSSION

Over the past century, a number of synthetic antiviral agents had been and developed, but lack of efficiency, drug resistance and toxicity are still the major hindrances to gaining successful therapeutic outcomes in many instances**.** Thus there is a need to search for new antiviral agents for future therapy and there is no doubt that medicinal plants can serve as a potential source for antiviral agents.[24]

Different kinds of essential oils were tested, from those with a typical monoterpene hydrocarbon pattern (*Eucalyptus globulus*) to those characterized by the presence of aldehydes (*Cymbopogon citratus* and *Pelargonium graveolenes),* phenolics (*Mentha piperita* and *Thymus vulgaris*) and alcohols (*Melaleuca altrenfolia*). The variability in cytotoxcity results of essential oils where the maximum non-toxic dose ranged from100 to 600ppm was related to the difference in major constituents of each oil. The essential oils which contained phenolic group compounds were more cytotoxic, where the maximum non toxic dose ranged from 100 to 200 ppm. This could be due to the presence of hydroxyl groups, which are directly related to the increase of intracellular oxygen reactive species, possibly leading to cell damage.[25] Of the three identified ethanolic extracts evaluated in the present study,

Glycyrrhiza glabra exhibited high cytotoxic activity this result was in agreement with the finding of.[26]

Antiviral activity against HSV-1: The results of this study showed that essential oil of *Mentha piperita, Cymbopogon citrates, Melaleuca alternifolia* and *Thymus vulgaris* had antiviral activity against HSV-1when the virus was treated with these oils prior to adsorption, but not after penetration into the Vero cell. These results are consistent with the findings of[28,19,27,29] who reported that essential oils were effective and acted only as virucidal against herpes virus and found their activity ranged from moderate to poor.[30,31] suggested that these virucidal essential oils directly inactivate herpes virus and might interfere with virion envelope structures or mask viral structures that are necessary for adsorption or entry into host cells**.** This suggestion was in line with the explanation of[29] who reported that virucidal essential oils denature viral structural proteins or glycoproteins, thus, infectivity of virus particles is completely lost. Also,[32] studied the effect of *Oregano* and *Clove* essential oil on HSV-1 by electron microscopic examination and they demonstrated that the envelope of HSV-1 was disrupted when treated with their essential oils. These findings were also confirmed by the study of[33] who reported that Adenovirus was not affected by *Eucalyptus* essential oil due to the lack of a viral envelope**.** Essential oils seem to be mostly efficient on cell-free virus but have limited effects on virus replicating in cells and on the cell-to-cell spread of the virus. Since essential oils are able to inhibit acyclovir-resistant HSV-1 isolates, the mechanism of interaction between these compounds and acyclovir with HSV could be different. Acyclovir inhibits virus replication by interference with the DNA polymerase inside the cell, whereas essential oils probably inactivate HSV before entering the cell. Viral resistance to acyclovir represents a particular problem; the prevalence of resistance in acyclovir-treated immunocompromised individuals is ~4–7%. Therefore other antiherpetic agents that are effective for viral mutants resistant to current antiviral agents are of great interest for topical treatment.[30]

The results of the present study showed that ethanolic extract of *Glycyrrhiza glabra* showed virucidal and anti-replication effect against HSV-1virus. This result was in line with the data of[26,34,35] who reported that HSV-1 and 2, Hepatitis A, B, C; HIV; Epstein Barr virus (EBV); influenza virus; cytomegalovirus (CMV); and cancer were inactivated by glycyrrhizin. Also[36] demonstrated in a case report that a two-percent topical glycyrrhizic acid cream (carbenoxolone sodium) applied six times daily in 12 patients with acute oral herpetic

infections(HSV-1) resolved pain and dysphagia within 24-48 hours. Moreover, the accompanying ulceration and lymphadenopathy gradually healed within 24-72 hours.

It was found in the present study that *Plantago major* showed weak antiviral effect, same results were reported by.[37] In contrast[38] reported that hot water extracts of *P. major* and *P. asiatica* possessed a broad-spectrum of antileukemia, anticarcinoma and antiviral activities, as well as activities which modulate cell-mediated immunity and secretion of interferon which may explain the reason for the popular use of P. major in the traditional Chinese medicine for treating infectious diseases.[39]

On studying the essential oils of *Ocimum basilicum; Pelargonium graveolens* and *Zizyphus spina- christi,* they showed no antiviral activity against HSV-1as regards virucidal, anti-adsorption and anti-replication effect. Similar findings were reported by.[27] However[40] reported that *Zizyphus spina -christi* exhibited significant antiviral properties against HSV- 1.

Antiviral activity against HAV: The present study showed that HAV (RNA virus) is less susceptible to essential oils than HSV-1(DNA). It was found that ethanolic extract of *Glycyrrhiza glabra* was the only extract effective against HAV. Similar findings were reported by[26,30,32] who reported that when Glycyrrhizin was tested against RNA viruses like Chandripura virus, Measles virus, Polio vaccine viruses type 1,2 and 3, Polio wild type viruses 1,2 and 3 as well as DNA viruses like HSV- 1 and 2 viruses *in vitro*. It inhibited the DNA virus plaque formation at lower concentrations (0.608mM) while the RNA viruses were inhibited at higher concentrations (1.216mM)**.** Also, *in vitro* research with glycyrrhizin and a human hepatoma cell line demonstrated that glycyrrhizin completely suppressed the expression of HAV antigen. In comparison to ribavirin, glycyrrhizin proved to be 10 times more potent at reducing infectivity of HAV, as measured by reduction in viral titers. It also exhibited five-fold cell selectivity greater than ribavirin, so it was less cytotoxic to the hepatoma cells. These results indicate glycyrrhizin may be a potential therapeutic adjunct in fighting HAV infections.[41]

On the other hand, *Lemongrass, Peppermint* and *Thyme* showed moderate effects against HAV while, *Basil, Geranium, Plantago, Zizyphus* and *Eucalyptus* showed no activity against it. Similar finding were reported by[42] who found that polio virus (RNA virus) was more resistant to many aqueous extracts.

Antioxidant activity: the antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants. Moreover, essential oils by scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline, as there is increasing evidence suggesting that these diseases may result from cellular damage caused by free radicals.[43] Free radical-scavenger effectiveness in the present study was in the following descending order: *Thymus vulgaris*> *Mentha piperita* > *Melaleuca alternifolia*> *Cymbopogon citratus* > *Eucalyptus globules* > *Pelargonium graveolens* > *Ocimum basilicum*. The different antioxidant activities could be related to differences in major constituents and the chemical complexity of essential oils. Different kinds of essential oils were tested, from those with a typical monoterpene hydrocarbon pattern (*Eucalyptus globulus*) to those characterized by the presence of aldehydes (*Cymbopogon citratus* and *Pelargonium graveolenes*), phenolics (*Mentha piperita* and *Thymus vulgaris*) and alcohols (*Melaleuca altrenfolia*). *Thyme* followed by *Peppermint* essential oil showed the strongest antioxidant activity. This result is in agreement with the concept that the structural feature required for a strong free radical-scavenging activity may be associated with the total phenolic content of the essential oil.[44,45,46 ,47] The potency of antioxidant activity of tea tree oil (*Melaleuca alternifolia)* was found to be comparable to that of the common synthetic antioxidant butylated hydroxytoluene (BHT). This suggests that tea tree oil might become a useful antioxidant agent.[48] The main antioxidant activity from the essential oils might be due to the ability of the alcohol, phenolic and aldehydric compounds in the oils donating hydrogen to free radicals and thus stopping the chain reaction of lipid oxidation at the initial step.[49] In this context, *C.citratus* essential oil, gave interesting results, being one of the best performing extracts in terms of ability to neutralize free radicals and prevent unsaturated fatty acid oxidation.[50] Analysis of geranium essential oil showed citronellol and transgeraniol as the major constituents which are known to possess antioxidant and anticancer properties.[51] The present work showed that basil essential oil had weak antioxidant activity in comparison with the others essential oils. This finding in line with the observation of.[52]

CONCLUSION

- The present study showed that Herpes simplex virus was more sensitive than Hepatitis A virus towards plant extracts.
- Essential oils seem to be mostly efficient on cell free virus (virucidal) but have limited effects on viruses replicating in cells and on the cell-to-cell spread of the virus with the

exception of ethanolic extract of *Glycyrrhiza glabra* which had anti-replication activity against the two viruses.

- Results of antioxidant activity (radical scavengers) showed that all essential oils tested showed considerable activity. *Thymus vulgaris* was the most effective.

RECOMMENDATIONS

- The results of this study may open the way to give more attention to the use of essential oils or ethanolic extracts as potentially cheaper alternative antiviral agents and posing little threat to induce resistance. They can be applied as topical therapeutic agents in the treatment of recurrent herpes infection and can be used also as food preservative to control hepatitis outbreaks.
- It is important to perform skin irritation tests and the efficacy of these oils and ethanolic extracts must be further studied in an *in vivo* model.
- The antioxidant activity of essential oils may open the way to give more attention to their use in controlling diseases and as food preservatives to control lipid peroxidation and food deterioration, to overcome human diseases caused by free radicals.

REFERENCES

1. Cos P, Vlietinck AJ, Vanden Berghe D and Maesa L (2006). Anti-infective potential of natural products: How to develop a stronger in vitro ‘proof-of-concept’. *Journal of Ethnopharmacology*, 106: 290–302.
2. Jassim SAA and Naji MA (2003). Novel antiviral agents: a medicinal plant perspective. *Journal of Applied Microbiology*, 95: 412-427.
3. Kott V, Barbini L, oz J de D, Vivot E, Crua es J, Martino V, Ferraro G,Cavallaro L and Campos R (1999). Antiviral activity in Argentine medicinal plants. *Journal of Ethnopharmacology*, 64: 79-84.
4. Rates, S.M.K. (2001). Plants as source of drugs. *Toxicon*, 39: 603-613.
5. Cordell GA (1995). Changing strategies in natural products chemistry. *Phytochemistry*, 40: 1585-1612.
6. Cowan, M.M., (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review*, 12: 564-582.
7. Astani A., Jürgen Reichling and Paul Schnitzler (2011).Screening for Antiviral Activities of Isolated Compounds from Essential Oils Evidence-Based Complementary and Alternative Medicine. 8 pages.

8. Whitley, R. J. (2001). Herpes Simplex Viruses. Third edition ed. *In* "Fields Virology" 2: 2461-2509. 2 vols. Lippincott-Williams &Wilkins, Philadelphia, PA.
9. Costa-Mattioli, Mauro Anna Di Napoli, Virginie Ferré, Sylviane Billaudel, Raul Perez-Bercoff and Juan Cristina (2003).Genetic variability of hepatitis A virus J Gen Virol, 84: 3191-3201; DOI 10.1099/vir.0.19532-0.
10. Halliwell,B and J. M. Gutteridge,(1989). Free Radicals in Biology and Medicine, Clarendon Press, Oxford, UK.
11. Halliwell, B(1997). "Antioxidants and human disease: a general introduction," Nutrition Reviews, 55(1): S44–S52, 1997.
12. Peschel W, Sanchez, R.F, Dieckmann W, Plescher A, Gartzia I, Jimenez D, Lamuela RR, Buxaderas S and Codina C (2006). An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. Food Chem., 97: 137-150.
13. Bozin, B. N. Mimica-Dukic, N. Simin, and Anackov, G. (2006) Characterization of the volatile composition of essential oils of some *lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils ,*Journal* of *Agricultural and Food Chemistry*, 54(5): 1822–1828.
14. Wei, A. and Shibamoto, T. (2007). Antioxidant activities and volatile constituents of various essential oils," *Journal of Agricultural and Food Chemistry*, 55(5): 1737–1742.
15. Alyne V. Barros1, Aline O. da Conceição, Isabela C. Simoni1, ClariceW. Arns3, Maria Judite B. and Fernandes1 (2010). Mechanisms of antiviral action of seeds from *Guettarda angelica* Mart. against bovine and swine Herpesvirus in vitro. *Virus reviews and reaserch, 15, Nr. 2:* 78.
16. Farag R.S., Daw Z.Y., Hewed F.M. and El-Baroty G.S.A. (1989). Antimicrobial activity of some Egyptian spice oils. Journal of Food Protection, 52: 665–667.
17. Kudi,A.C and S.H.Myrint, (1999).Antiviral activities on some Nigerian medicinal plants extract.J.of Ethnopharmacol, 68: 289-294.
18. Cromean, T., Sobsey M.D., and Field H.A (1987). Development of a plaque assay for a cytopathic rapidly replicating isolate of hepatitis A virus J.medical virology, 22: 45-56.
19. Schuhmacher A, Reichling J and Schnitzler P. (2003). Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 in vitro. Phytomedicine, 10: 504–510.
20. Zhang J, Zhan B, Yao X, Gao Y and Shong J (1995). Antiviral activity of tannin from the pericarp of *Punica granatum* L. against genital herpes virus *in vitro*. Zhongguo Zhong Yao Za Zhi, 20(9): 556-558, 576.

21. Amoros M, Lurton E, Boustie J and Girre L (1994). Comparison of the antiherpes simplex virus activities of propolis and 3-methyl-But-2Enyl cafferate. J. Natural products, 57(5): 644-647.
22. Dorman, H.J.D., Deans, S.G., Noble, R.C. and Surai, P., 1995. Evaluation in vitro of plant essential oils as natural antioxidants. Journal of Essential Oil Research, 7: 645–651.
23. Aeschbach. R, J. Loliger, B.C. Scott, A. Murcia, J. Butler and Haliwell, B. (1994). Antioxidation actions of thymol, carvacrol 6-gingerol, zingerone and hydoxytyrosol, Food Chemistry Toxicology., 32: 31–36.
24. Richman, D.D (2006).Antiviral drug resistance .Antiviral research, 71: 117-121.
25. Matsuo, M., Sasaki, N., Saga, K. and Kaneko, T., (2005). Cytotoxicity of flavonoids toward culture normal human cells. Biol. Pharm. Bull, 28: 253–259.
26. Badam-L. (1997). *In vitro* antiviral activity of indigenous glycyrrhizin, licorice and glycyrrhizic acid (Sigma) on Japanese encephalitis virus. Journal-of Communicable Diseases, 29: 2, 91-99; 13.
27. Minami M, Kita M, Nakaya T, Yamamoto T, Kuriyama H and Imanishi J(2003).The inhibitory effect of essential oils on Herpes simplex virus type 1replication in vitro. Microbiol Immunol, 47: 681–684.
28. Schnitzler, P., K. Scho¨n, and Reichling. J. (2001). Antiviral activity of Australian *Tae tree* oil and *Eucalyptus* oil against herpes simplex virus in cell culture. Pharmazie, 56: 343–347.
29. Koch C, Reichling J, Schneele J and Schnitzler P:(2008). Inhibitory effect of essential oils against herpes simplex virus type 2. Phytomedicine, 15: 71–78.
30. Schnitzler, P Christine Koch, and Jürgen Reichling (2007).Susceptibility of Drug-Resistant Clinical Herpes Simplex Virus Type 1 Strains to Essential Oils of Ginger, Thyme, Hyssop, and Sandalwood Chemotherapy, 51(5): 7.
31. Astani A., Jürgen Reichling and Paul Schnitzler (2011). Screening for Antiviral Activities of Isolated Compounds from Essential Oils Evidence-Based Complementary and Alternative Medicine. 8 pages.
32. Reichling, J. P. Schnitzler, U. Suschke, and R. Saller, (2009). "Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties—an overview," *Forschende Komplementarmedizin*, 16(2): 79–90.
33. Cermelli, C. Fabio, A. Fabio, G. and Quaglio, P. (2008). Effect of eucalyptus oil essential on respiratory bacteria and viruses. *Current Microbiology*, 56(1): 89–92.

34. Fiore C, Eisnhut M and Krausse R (2008). Antiviral effect of *Glycyrrhiza* species. Phytother Res, 22: 141-148.
35. Okuno, T, Arai, K and Shindo M. (2004).Efficacy of interferon combined glycyrrhizin therapy in patients with chronic hepatitis C resistant to interferon therapy. Nippon Rinsho, 52(7): 1823-7.
36. Hirabayashi K, Iwata S, Matsumoto H, et al. (1991). Antiviral activities of glycyrrhizin and its modified compounds against human immunodeficiency virus type 1 (HIV-1) and Herpes simplex virus type 1 (HSV-1) in vitro. Chem Pharm Bull., 1991; 39: 112-115.
37. McCutcheon AR, Roberts TE and Gibbons E (1995). Antiviral screening of British Colombian medicinal plants. Ethnopharm., 49: 101-110.
38. Chiang L.C., Chiang W., Chang M.Y. and Lin C.C. (2003). *In vitro* cytotoxic, antiviral and immunomodulatory effects of *Plantago major* and *Plantago asiatica.* American Journal of Chinese medicine, 31: 225–234.
39. Chiang L.C; Chiang,W; Chang,M.Y and Lin,C.C (2002).Antiviral activity of *Plantago major* extracts and related compounds *in vitro*. Antiviral-Research, 55(1): 53-62.
40. Shahat,A.A., Pieters-L; Apers,S; Nazeif,N.M; Abdel-Azim- Berghe-D-vanden; Vlietinck-AJ and vanden-Berghe-D(2001). Chemical and biological investigations on *Zizyphus spina-christi* Pharmaceutical Sciences Department, National Research Centre, 12311 Dokki, Cairo, Egypt. Phytotherapy-Research, 15: 593-597.
41. Iino S, Tango T and Matsushima T (2001). Therapeutic effects of stronger neo-minopahgen C at different doses on chronic hepatitis and liver cirrhosis. Hepatol Res, 19: 31-40.
42. Abad, M. J., Guerra, A., Bermejo, P., Irurzun, A. and Carrasco, L., (2000). Search for Antiviral Activity in Higher Plant Extracts. J. Phytother., 14: 604-607.
43. Kamatou, G.P.P. and Viljoen, A.M.(2010). A review of the application and pharmacological properties of α- bisabolol and α-bisabolol-rich oils. *J. Am. Oil Chem. Soc.*, 87: 1-7.
44. Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V. and De Pasquale, A., (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chemistry, 89: 549–554.
45. Amiri, H. (2012). Essential Oils Composition and Antioxidant Properties of Three *Thymus* Species Evidence-Based Complementary and Alternative Medicine Volume 2012, Article ID 728065, 8 pages.

46. Dandlen, S.A.; Lima, A.S.; Mendes, M.D.; Miguel, M.G.; Faleiro, M.L; Sousa, M.J.; Pedro, L.G.; Barroso, J.G. and Figueiredo, A.C.(2010). Antioxidant activity of six Portuguese *Thyme* species essential oils. *Flavour Fragr. J.*, *25*: 150-155.
47. Sarikurkcu Cengiz, M. Sabih Ozer , Mustafa Eskici Bektas Tepe, Şendil Can and Ebru Mete (2010). Essential oil composition and Meteantioxidant activity of *Thymus longicaulis* .Food and Chemical Toxicology, 48: 1801-1805.
48. Noumi Emira, Mejdi Snoussi, Hafedh Hajlaoui1, Najla Trabelsi, Riadh Ksouri, Eulogio Valentin and Amina Bakhrouf (2011). Chemical composition, antioxidant and antifungal potential of *Melaleuca alternifolia* (tea tree) and *Eucalyptus globules* essential oils against oral *Candida* species. Journal of Medicinal Plants Research, 5(17): 4147- 4156.
49. Lertsatitthanakorn. P., S. Taweechaisupapong, C. Aromdee and W. Khunkittia, (2006). In vitro bioactivities of essential oils used for acne control. International Journal of Aromatherapy, 16: 43-49.
50. Saeio, K Wantida Chaiyana and Siriporn Okonogi (2011). Antityrosinase and antioxidant activities of essential oils of edible Thai plants. Drug Discoveries & Therapeutics, 5(3): 144-149.
51. Sofidiya, M.O., Jimoh, F.O, Aliero A.A, Afolayan AJ, Odukoya OA and Familoni O.B (2008). Antioxidant and Antibacterial properties of Lecaniodiscu-scupanioides. Res. J. Microbiol., 3: 91-98.
52. Wei, A. and Shibamoto,T. (2010). Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. J. Agr. Food Chem, 58: 7218-7225.