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Antibiofilm and Antiviral Potential of Leaf Extracts from *Moringa oleifera* and Rosemary (*Rosmarinus officinalis* Lam.)

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THE RISE of antimicrobial drug resistance due to biofilms and improper use of conventional drugs poses a major concern in medicinal fields. This work evaluates the antibiofilm and antiviral potential of leaf extracts from *Moringa oleifera* and *Rosemarinus officinalis* (Rosemary). Three different concentrations (20, 40 and 60 mg/ml) of *M. oleifera* leaves aqueous extract were investigated for their inhibitory potential on initial cell attachment of *Staphylococcus aureus* cells to the microtiter plate well surface. The concentration (60 mg/ml) resulted in 28.57% inhibition of biofilm formation as compared to 4.96% inhibition in the presence of 20 mg/ml of the same extract. In contrast,*S. aureus* was more susceptible to rosemary methanol extract with 41.30% inhibition using 10 mg/ml of the extract. Moreover, at non cytotoxic concentration, the inhibitory effect of *M. oleifera* and rosemary extracts have been separately evaluated against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). The results indicated that 200 µg/ml and 100 µg/ml for *M. oleifera* and rosemary extracts, respectively, were considerably safe for Vero cells as cell survival was above 90%. In addition, the percentage of inhibition was 43.2 and 21.4% for HSV-1and HSV-2, respectively by *M. oleifera* extract while rosemary caused 18.9% inhibition for HSV-1 and no inhibitory activity against HSV-2 was observed.Thus, results uncovered inhibitory potential of *M. oleifera* and rosemary leaf extracts on initial attachment of *S. aureus* to surfaces towards biofilm formation. In addition, *M. oleifera* extract was more effective than rosemary as antiviral agent against herpes simplex viruses.

Keywords: Antibiofilm, Antiviral, Plant extract, *Staphylococcus aureus*, HSV

Introduction

Biofilms are structural components including a community of bacterial cells within a self-produced polymeric matrix and adhering to an inert or living surface (Costerton et al., 1999). The key features of biofilms are the elevated resistance to antimicrobials than the sessile cells making their elimination a big challenge (Simões et al., 2011). Being important in human diseases, the number of biofilm-associated infections increases dramatically (Davies, 2003). Biofilm infections of *Staphylococcus aureus*, especially the medical device-associated infections are difficult to be eradicated using most of the currently available treatments (Lynch & Robertson, 2008).

The increase of biofilm resistance to traditional treatments such as antibiotics or chemical preservatives and their associated adverse side effects triggers a great interest to search for safer alternatives (Ahmad et al., 2014; Onsare & Arora, 2015). Plant-based antimicrobials gain much publicity because of their safety and long history of use. One of these plants is *Moringa oleifera* (Stohs & Hartman, 2015). The anti-inflammatory, antibacterial, and antifungal activities of *M. oleifera* were well characterized (Koheil et al., 2011; Oluduro, 2012 and Peixoto et al., 2011). In addition, *Rosmarinus officinalis* (Rosemary) has been recognized as an important member of medically important plants (Petiwala et al., 2014). The antioxidant and antimicrobial properties of rosemary extracts has been well-documented (Bubonja-Sonje et al., 2011).

The extracts derived from seeds, leaves, roots and fruits of medicinal plants exhibited antiviral activities against RNA and DNA viruses (Kanekiyo et al., 2008 and Todorov et al., 2015). A large number of bioactive plant constituents, such as phenolics and flavonoids found in leaves have been reported to be active anti-herpetic agents (Khan et al., 2005).

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Herpes simplex viruses are large enveloped double stranded DNA viruses with icosahedral symmetry, categorized into Herpes simplex virus type 1 (HSV-1/ oral herpes) and herpes simplex virus type 2 (HSV-2/ genital herpes) of the family of Herpesviridae. This family of viruses has been known to establish lifelong latency causing recurrent episodes of the disease. Both viruses cause infections that are mostly asymptomatic but can cause painful blisters or ulcers at the site of infection of the nervous system around the lips, in the eyes, in the mucous membrane of the oral cavity and also in the genitals (Kenneth & Ray, 2004). Transmission occurs by contact with secretions from an infected person with either overt infection or asymptomatic excretion of virus. Acyclovir and penciclovir are now drugs for treatment of HSV infection but viral resistance against acyclovir has become an arising problem in the therapy of herpes simplex viruses' diseases especially for the treatment of immune-compromised patients (Villarreal, 2003). Due to resistance of pathogens to chemical drugs, the search for biological active extracts based traditionally used plants is still relevant. Investigations for antiviral activity of some plant leaves extracts represent an important step in finding an alternative viral disease treatment. Crude extracts of plant materials are a promising source of systemic broad spectrum antiviral that may cause less damage to host cells than do pharmaceuticals (Barakat et al., 2010 and Kim et al., 2016). Leaves' extracts of *M. oleifera* are mostly used as food items, folk traditional medicine of Africa and South Asia and for a variety of medicinal purposes (Jung, 2014).The inhibitory properties of *M. oleifera* have been stated against much viral diseases (Murakami et al., 1998 and Nworu et al., 2013). Rosemary is an aromatic evergreen herb shrub that is widely distributed in the Mediterranean region. Polyphenols derived from aromatic plants and spices have been used as natural antiviral agents (Cushnie & Lamb, 2005; García et al., 2003 and Khan et al., 2005), specifically, the flavonoids including galangin, quercetin, procyanidin, pelargonidin and procyanidin C-1 (Amoros et al., 1992; Meyer et al., 1997 and Shahat et al., 2002). The antiviral effect of these substances is greatest when used before virus adsorption (Lyu et al., 2005 and Shahat et al., 2002). Based on the promising safety, antimicrobial and antiviral characteristics of *M. oleifera* and rosemary against pathogens, our work aimed here to explore their antibiofilm and antiviral potential against the biofilm forming *S. aureus* and herpes simplex viruses.

Materials and Methods

Plant extracts preparation

The experiments used leaves of *M. oleifera* and rosemary which were collected from botanical gardens of Faculty of Agriculture, Ain-Shams University, Egypt. For preparation of aqueous extracts; 20, 40 and 60 mg of *M.oleifera* dry leaves were homogenized into a fine powder in a mortar and pestle device very gently and dissolved in 1 ml of sterile distilled water. For rosemary, dry powdered leaves (10, 20 and 40 mg/ml) were used for the biofilm experiment. Dry powdered plant leaves were extracted in soxhlet apparatus using petroleum ether (2:2.5 volumes) for 8 h. After extraction, the solvent was evaporated using rotary vacuum evaporator to give a concentrated extract at 60˚C in a water bath, then dried aseptically and subjected to gas chromatography-mass spectroscopic (GC-MS) analysis.

Inoculum preparation

Initial active bacterial inoculum for the biofilm experiment was prepared as described earlier (Arora & Kaur, 1999). A loopful of well-isolated colonies of *S.aureus* (obtained from Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Egypt) was transferred into 5 ml of nutrient broth (Oxoid, UK) and incubated for16 h at 37°C to obtain the cells at exponential phase.

Evaluation of antibiofilm potential

Attachment of free cells onto a surfaceto form sessile cells is the first stage towards biofilm development. Disruption of the initial attachment process of the cells is the starting point of finding promising antibiofilm agents. The antibiofilm formation activity of *M. oleifera* and rosemary leaf extracts was assessed as previously described (Jadhav et al., 2013). Two sets labeled test agents (*M. oleifera* and rosemary) along with their respective experimental control (broth medium) were prepared in microtitre plate. A series of inhibitory concentrations of test antibiofilm agents as previously described (Elhariry et al., 2014 and Oluduro, 2012) were prepared. In each case, 100 µl of the test antibiofilm agent prepared in different inhibitory concentrations (*M. oleifera* extract; 20, 40 and 60 mg/ml; rosemary extract 10, 20, 40 mg/ml) was added in triplicate to the corresponding wells of sterile flat-bottomed 96-well microtitre plate (Sigma Aldrich, USA) except the negative controls (untreated cells). Equal volumes of the bacterial cultures were added into wells in all the

sets (except the experimental control in which same volume of broth medium was added instead) so as to have a final volumeof 200 µl per well. The microtitre plates were sealed and incubated for 16 h at 37°C. The inhibition of initial cell attachment in the presence of the test agents corresponds to the biofilm biomass as compared to negative control was established using crystal violet assay. The results have been expressed as percentage of inhibition.

The estimation of biofilm biomass was carried out after the treatment periods in preceding experiments. Quantification of the biomass was carried out by crystal violet (CV) assay (Djordjevic et al., 2002) with slight modifications. The evaluation is based on the fact that the dye penetrates and stains cell membranes of attached cells thereby providing information of their density. The amount of stain absorbed is directly proportional to the amount of biofilm on surfaces. After incubation of treated test organisms, the culture medium from each well was discarded and washed two times with sterile distilled water to wash away any loosely adhered cells. The air-dried wells were further dried in the oven at 60°C for 40 min and then stained with 150µl of 0.1% crystal violet before incubating at room temperature for 20 min. After incubation, the wells were again washed two times with sterile distilled water to remove unabsorbed stain. For estimating biofilm density, the absorbance was determined at 595 nm using an automated microplate reader (Sun Rise –TECAN. Inc.® USA). The mean absorbance ($OD_{595\ nm}$) of test organisms was determined and the percentage inhibition was calculated using the following formula (Equation 1):

Percentage inhibition = 100 – ((OD_{595nm}test for positive control well /OD_{595nm}negative control well) x 100)

Herpes simplex virus

Herpes simplex type 1 and type 2 virus stocks were prepared by infecting cell lines in plastic flasks at 37°C. The cells were checked by microscopy for cytopathic effects (CPE). At 75% CPE, the medium was decanted and stored at -70 ºC. HSV-1 and HSV-2were propagated and quantified at 50% tissue culture infective dose ($TCID_{50}$) by end point dilution (Flint et al., 2015).

Cell line

African green monkey kidney cell lines (Vero cells) were obtained from European Collection of Cell Cultures (ECACC). The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) (Life Technologies, Inc.) supplemented with 10% heat-inactivated FBS, 2 mM glutamine, and 1% gentamycinat 37ºC with 5% CO_2.

Cytotoxicity assay

Cytotoxicity of each extract was monitored on Vero cells. Vero cells were propagated in DMEM supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. The cells were seeded onto 96-well microtitre plates (Falcon, NJ, USA) at 10^4cells/ml. Fresh maintenance medium containing different concentrations of the *M. oleifera* and rosemary leaves extracts were added after 24 h of seeding. Control cells were incubated without test material. The plates were incubated in a humidified incubator with 5% CO_2 at 37ºC for 48 h. Each concentration of test extracts was loaded into six wells. Detectable alterations in morphology of the cells were inspected daily. By MTT assay, the optical density was measured at 490 nm using ELISA reader (Vmax Molecular Devices) to determine the number of viable cells, and the viability percentage was calculated as $[1-(OD_t/OD_c)]$ x100% where OD_t is the mean optical density of wells separately treated with the *M. oleifera* and rosemary leaves extracts and OD_c is the mean optical density of untreated cells (Mosmann, 1983).

Evaluation of antiviral activity

The antiviral activity of the tested aqueous extract derived from *M. oleifera* and methanol extract from rosemary leaves was separately evaluated by MTT method (Mosmann, 1983). Briefly; monolayers of Vero cells were seeded into 96-well culture plate at 10^5 cells/ml with a volume of 100 µl/well. After 24 h of incubation at 37ºC in a CO_2 incubator, 100 µl of 100 $TCID_{50}$ of the herpes simplex type 1 or type 2 virus was added, and the infected cells were incubated for another 2 h. The medium is removed after incubation, and 125 µl of experimental leaves extract is added to the first row in triplicate wells, all other wells having 100 µl of DMEM with 2% fetal calf serum. The test material in the first row of wells is then diluted serially 1:5 throughout the remaining wells by transferring 25 µl. After further incubation at 37ºC with 5% CO_2 for 48 h, MTT was added. The culture was incubated for 4 h to allow the production of formazan, and then 100 µl of acidified isopropanol (0.04 N HCl in isopropanol)

was added to each well to dissolve any formazan produced. The *M. oleifera* and rosemary leaves extracts were evaluated using a minimum of 6 replicates. The amount of formazan produced is directly proportional to the number of surviving cells. A positive growth control, 6 wells/plate, contained non-infected Vero cells grown in the presence of 0.2% DMSO. A viral death control, 6 wells/plate, contained viral infected cells. The plate was measured at 490 nm. Data manipulated by computer outfitted with the Softmax Pro® software and linked to the plate reader. The A_{490} values, corrected for the cytotoxicity exerted by test extracts (as determined in non-infected cultures), were used to calculate the percent cell viability (Fernandez-Pol et al., 2001).

Viral inhibition rate was estimated using the following formula:

Viral inhibition percentage = $[(OD_{tv}-OD_{cv})/(OD_{cd}-OD_{cv})]\times100\%$

where OD_{tv}, OD_{cv} and OD_{cd} are the absorbance of test extracts with virus infected cells, the absorbance of the virus control and the absorbance of the cell control, respectively.

Analysis by gas chromatography-mass spectrometry (GC-MS)

The chemical composition of the aqueous extract of *M. oleifera* and methanol extracts of *R. officinalis* were determined using GC-MS to identify the probable bioactive antibiofilm and antiviral constituents. The GC-MS analysis of the crude extracts were performed using Perkin Elmer system (GC clarus 600, USA) equipped with an AOC-20i autosampler. Gas chromatograph was interfaced to a mass spectrometer (GC-MS) instrument with Elite-5MS fused silica capillary column (30.0 m× 0.25 mm ID, 250 μmdf). Detection was operating in electron impact mode with ionization energy of 70 eV with helium (99.99%) as a carrier gas at flow rate of 1ml/min. The injection volume of 1 μl of sample was employed (split ratio of 10:1) and the injector temperature was at 240°C. The oven temperature was 60°C with an increase of 10°C/min to 300°C/min for 6 min. The components of the extract were identified by the retention time of the chromatogram peaks and by their mass spectra. The identities of the main components were confirmed by comparison of their retention time with that of reference compounds. The percentage of each compound was calculated as the ratio of the peak area to the total chromatographic area.

Results

The effect of *M. oleifera* and rosemary extracts on initial cell attachment of *S. aureus* towards biofilm formation was clearly observed. The inhibitory potential against initial cell attachment of rosemary was stronger than *M. oleifera* extract. A decrease in the biomass ($OD_{595nm)}$ of *S. aureus* was observed by the gradual increase of *M. oleifera* extract concentrations of which the concentration (60 mg/ml) resulted in 28% inhibition of biofilm formation as compared to 4.96% and 13.97% inhibition in the presence of 20 and 40 mg/mlof the same extract, respectively (Table 1). On the other hand, *S. aureus* was more susceptible to rosemary extract with 41.30% inhibition using 10 mg/ml of the crude extract. Increasing the concentration of rosemary extract was surprisingly associated with less inhibitory potential 22.67% and 23.91% using 20 and 40 mg/ml, respectively (Table 1). Results showed encouraging inhibitory potential of *M. oleifera* and rosemary leaf extracts being the highest promising at lower concentration against *S. aureus* biofilm formation.

Cytotoxicity assessment of two extracts derived form *M. oleifera* and rosemary leaves showed survival of Vero cells by 92 and 91% at concentration 200 μg /ml and 100 μg/ml, respectively (Table 2). Cell survival of Vero cells was above 50% using the former concentrations while at higher concentration, cell survival was below 50% (Data not shown).

The *M. oleifera* and rosemary extracts were investigated for their antiviral activity against herpes simplex viruses. Antiviral assay of *M. oleifera* aqueous extract at concentration of 200 μg/ml revealed inhibition percentage of 43.2% and 21.4% for herpes simplex type 1 and type 2, respectively. On the other hand, using 100 μg/ml of rosemary extract, inhibitory activity was 18.9% for herpes simplex type 1. Moreover, at the same concentration of rosemary extract, no antiviral activity against HSV-2 was observed. Cell survival percentages and antiviral potential mediated by aqueous *M. oleifera* and methanolic extract of rosemary leaves (Table 2).

TABLE 1. Antibiofilm potential of *Moringa oleifera* and rosemary leaf extracts against *S. aureus.*

M. oleifera **extract (mg/ml)**	**Mean of OD_{595nm}**	**% Inhibition**	**Rosemary extract (mg/ml)**	**Mean of OD_{595nm}**	**% Inhibition**
60	0.076 ± 0.007	28.57	40	0.081 ± 0.007	23.91
40	0.092 ± 0.006	13.97	20	0.083 ± 0.033	22.67
20	0.102 ± 0.010	4.96	10	0.063 ± 0.003	41.30
0 (control)	0.0107 ± 0.002	NA	0 (Control)	0.0107 ± 0.002	NA*

*Denotes for not applicable

TABLE 2.Cytotoxicity and antiviral activity of *Moringa oleifera* and rosemary leaf extracts against Herpes simplex viruses.

Plant name	**Extract**	**Concentration µg/ml**	**%Vero Cell survival**	**%Inhibition**	
				HSV-1	**HSV-2**
M. oleifera	Aqueous	200	92	43.2	21.4
Rosemary	Methanol	100	91	18.9	ND*

*ND: Not determined

The GC –MS results for *M. oleifera* and rosemary leaf extracts are presented in Table 3. Only the compounds with relative occurrence > 1% are reported. The *M. oleifera* leaf extract had 17 compounds accounting for 83.26% of the total chemical composition of the extract. D.L.Alpha –Tocopherol was the compound found in highest concentration (11.89%) followed by 2, 4, 6- cycloheptatrien-1-one,3,5-bis-trimethylsilyl (9.68%), Trimethyl (4-tert-butyphenoxy) silane (7.51%), 1-Nonene, 4,6,8- trimethyl (7.0%), Oxirane, Hexadecyl (6.70%) and others as shown in Table 3.

For rosemary, the compound found in the highest concentration was Ferruginol (4.53%) as demonstrated in Table 3. Also, Camphor (3.88%), Cineole (2.66%) and Verbenone (2.50%) existed in considerable amounts and all in addition to Ferruginol accounted for 15.13% of the whole chemical composition of the extract (Table 3).

TABLE 3. Major chemical compounds identified from *Moringa oleifera* and rosemary leaf extracts and their percentage occurrence (> 1%).

Name of compound	Molecular formula	Occurrence (%)
Moringa oleifera		
Dibutyl phthalate	$C_{16}H_{22}O_4$	1.66
4- Dodecanol	$C_{12}H_{26}O$	1.37
Hexanedioic acid, bis (2-ethylhexyl)	$C_{22}H_{42}O_4$	1.36
Z6, Z9- Pentadecadien-1-ol	$C_{15}H_{28}O$	3.20
Heptane, 2,2,3,3,5,6,,6-Heptamethyl	$C_{14}H_{30}$	3.86
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	4.34
Hentriacontane	$C_{31}H_{64}$	3.26
1-Nonene, 4,6,8- trimethyl	$C_{12}H_{24}$	7.0
1-Hexanol, 2-ethyl-2-propyl	$C_{11}H_{24}O$	6.04
Squalene	$C_{30}H_{50}$	5.34
Trimethyl (4-tert-butyphenoxy) silane	$C_{13}H_{22}OSi$	7.51
Tetracontane-1,40-diol	$C_{40}H_{82}O_2$	2.22
Tetrapentacontane and Dotriacontane	$C_{32}H_{56}$	4.16
D.L.Alpha -Tocopherol	$C_{29}H_{50}O_2$	11.89
Oxirane, Hexadecyl	$C_{18}H_{36}O$	6.70
Beta- Amyrin	$C_{32}H_{52}O_2$	3.64
2,4,6- cycloheptatrien-1-one,3,5-bis-trimethylsilyl	$C_{13}H_{22}OSi$	9.68
Total		83.26
Rosemary		
Cineole	$C_{10}H_{18}O$	2.66
Camphor	$C_{10}H_{16}O$	3.88
Borneol	$C_{10}H_{18}O$	1.56
Verbenone	$C_{10}H_{14}O$	2.50
Ferruginol	$C_{20}H_{30}O$	4.53
Total		15.13

Discussion

Plants represent a sustainable source of antibiofilm agents with different modes of action and properties (Ahmad et al., 2014). Phytochemical screening of *M. oleifera* aqueous extract revealed several classes of secondary metabolites such as alkaloids, polyphenols (such as Tocopherol), flavonoids and terpenes (such as Beta-amyrin). Several molecules belonging to these classes were found to be effective against pathogenic microorganisms (Ndhlala et al., 2013 and Viera et al., 2010). The presence of these classes can account for antibiofilm potential of *M. oleifera* leaf extract (Dzotam et al., 2016). Similarly, the antibiofilm potential of flavonoids extracted from *M. oleifera* seed coat was quite encouraging (Onsare & Arora, 2015). In addition, flavonoids such as kaempferol present in citrus was proved to exhibit anti-quorum sensing activity by interfering with the acyl-homoserine lactones leading to inhibition of biofilm formation (Vikram et al., 2010). In leaf extract of *M. oleifera*, total flavonoids were estimated to be present in considerable quantities (Vongsak et al., 2013). In this study, the potential of bioactive constituents of *M. oleifera* leaf extract to inhibit initial cell attachment to surfaces is dependent on concentration in a similar way to previous studies (Onsare & Arora, 2015). This gives credence to our work where increasing concentration of the extract resulted in decrease of cell biomass. However, it is suggested to combine *M. oleifera* extract with other known antibiofilm safe phytocompounds to maximize their potential to render unfavorable conditions for initial attachment of bacterial cells to surfaces. In general, inhibition of such attachment to surfaces may be the starting point to combat formation of biofilm. On the other hand, rosemary extract exhibited promising antibiofilm potential against *S. aureus*. The antimicrobial activities of rosemary against a wide range of microbial pathogens such as *S. epidermidis*, *Escherichia coli* and *Candida albicans* has been reported (Abramovič et al., 2012). In addition, a synergistic antimicrobial activity of rosemary and galangal or lemon iron bark was observed when they are used in combination (Weerakkody et al., 2011). Intriguingly, seven culinary herbs caused at least 50% reduction of biofilm adhesion of some strains of *Listeria monocytogenes* but rosemary was one among three that inhibited preformed biofilms (Sandasi et al., 2010). The antibiofilm activity of rosemary extract is thought to be attributed to terpenes (such as Cineole, camphor and borneol) and phenolic constituents (such as Ferruginol) present in the extract (Weerakkody et al., 2011).

On the other hand, this study aimed to investigate the inhibitory effect of two different extracts from medicinal plants against herpes simplex virus using Vero cell line. Aqueous extract from *M. oleifera* showed moderate inhibitory activity against herpes simplex virus type 1 and weak inhibitory effect against herpes simplex virus type 2 at a non-cytotoxic concentration, these results were in accordance to previous studies (Anwar et al., 2007 and Lipipun et al., 2003).

Methanol extract derived from rosemary leaves showed a less inhibitory activity against herpes simplex virus type 1 and no effect on HSV-2 where as most HSV-2 strains are highly resistant to acyclovir due to a mutation previously reported in the viral DNA polymerase (Kriesel et al., 2005). The antiviral activity of the two plant extracts used in this work might be related to the presence of phenolic compounds such as ferruginol (Jung, 2014). The phenolic compounds are responsible for the inhibitory effects of lipid peroxidation, antiviral activity against replication of either RNA or DNA viruses, disability of the viral lipidic envelope and interfering with viral compounds required for adsorption and cell entry (García et al., 2003; Minami et al., 2003 and Vadlapudi et al., 2013). Aqueous extract from rosemary possesses antioxidant activity coupled with antiviral action (Mancini et al., 2009). In the same study, the experiments with HSV-1 replication in Vero cells, indicated inhibition of the virus in the range of 85 to 86 % in the presence or absence of both, natural and synthetic antioxidants (Mancini et al., 2009).

Our results demonstrated that aqueous extract of *M. oleifera* showed antiviral activity against HSV-1in Vero cells. These findings suggest that flavonoid compounds such as Beta-amyrin may result in decrease or inhibition of the yield of HSV-1 by an inhibition of viral DNA replication (Nolkemper et al., 2006).

There was no significant difference between acyclovir and *M. oleifera* extract in the delay of the development of skin lesions (Lipipun et al., 2003). Toxicity of *M. oleifera* extract was not observed in treated mice. Thus, this plant extract may be possible candidate as anti-HSV-1 agent.

Our data showed that rosemary extract only had a weak and no inhibitory effect on HSV-1 and HSV-2, respectively so it must be used in a combination with synthetic antioxidant compounds to improve its antiviral activity against herpes simplex viruses. Combination between natural extracts and therapeutic agents has been reported in many investigations. *Actium lappa*, *Calendula officinalis* and *Geranium robertianum* extracts may have different mechanisms of anti-HSV-1 action from acyclovir and thus the combination of acyclovir with herbal extracts might work synergistically (Corina et al., 1999).

The plants used for this study was found to contain phenols, flavonoids and terpenes as a phytochemicals which were demonstrated by GC-MS. So there may be a correlation between phenols and all these activities. Finally, the two plant extracts showed antiviral activity but *M. oleifera* extract was more effective than rosemary extract against herpes simplex viruses.

Our work emphasized *M. oleifera* and rosemary could be a rich source for bioactive components that can act as antibiofilm and antiviral agents against pathogens. Therefore, further studies are needed to isolate the bioactive constituents, exploring their mode of action, investigating their synergism when used in combinations and reveal their therapeutic potential in animal models.

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النشاط المضاد للغشاء الحيوى (البيوفيلم) والمضاد للفيروسات لمستخلصات اوراق المورينجا أوليفيرا وإكليل الجبل

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ان ارتفاع مقاومة الميكروبات للعقاقير الطبيه بسبب تكوين الأغشية الحيوية والاستخدام غير السليم لهذه العقاقير يشكل مصدر قلق كبير في المجالات الطبية. فى هذه الدراسه تم تقييم النشاط المضاد للأغشية الحيوية والمضاد للفيروسات لمستخلصات من أوراق المورينجا أوليفيرا وإكليل الجبل. تم اختبار قدرة ثلاثة تركيزات مختلفة (20 و40 و60 ملى جرام/مل) من المستخلص المائي لاوراق المورينجا أوليفيرا على تثبيط التصاق خلايا بكتيريا المكورات العنقوديه الذهبيه لاسطح wells طبق الاليزا. حيث أدى تركيز 60 ملى جرام/مل إلى تثبيط ٪28 من تكوين الغشاء الحيوى بالمقارنة بتركيز 20 ملى جرام/مل الذى ادى إلى تثبيط ٪4.96. في المقابل، وباستخدام تركيز 10 ملى جرام/مل من مستخلص اكليل الجبل كانت نسبه تثبيط تكوين الغشاء الحيوى ٪41.30.

وعلاوة على ذلك، تم تقييم التأثير المثبط لكل من مستخلصات أوراق المورينجا أوليفيرا و اكليل الجبل بشكل منفصل ضد فيروس الهربس البسيط 1 و2 (HSV-1, HSV-2)وأشارت النتائج الى أن 200 ميكروجرام/مل و 100 ميكروجرام/مل لكل من مستخلصات اوراق المورينجا أوليفيرا و اكليل الجبل على الترتيب كانت امنه لخلايا فيرو (Vero cells)، حيث كان نسبه بقاء الخلايا حيه أكثر من ٪90 و بنسبة تثبيط وصلت إلى 43.2 و٪21.4 لـ فيروس الهربس البسيط 1 و2 على الترتيب بمستخلص المورينجا أوليفيرا بينما كانت نسبه التثبيط ٪18.9 بواسطه مستخلص اكليل الجبل لفيروس الهربس البسيط 1 و لم يكن هناك نشاط مثبط ضد فيروس الهربس البسيط 2. وهكذا كشفت النتائج عن إمكانات مستخلصات المورينجا أوليفيرا و اكليل الجبل على تثبيط تكوين الاغشيه الحيوية لبكتيريا المكورات العنقوديه الذهبيه. وبالإضافة إلى ذلك، كان مستخلص المورينجا أوليفيرا اكثر فاعليه كعامل مضاد للفيروسات ضد فيروسات الهربس البسيط.