EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

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ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 17 No. 2 (2025)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.17 (2) pp.49- 71 (2025)*
DOI: 10.21608/EAJBSG.2025.451861

Egypt. Acad. J. Biolog. Sci., 17(2):49-71 (2025)

Egyptian Academic Journal of Biological Sciences
G. Microbiology
Res. Ethics No.: 03-2025-0023
ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Improving the Sensory Properties and Effectiveness of Facial Cream by Date Kernel Extract to Combat Skin Herpes Disease

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ARTICLE INFO
Article History
Received:30/7/2024
Accepted:8/9/2025
Available:12/9/2025

***Keywords*:**
HSV, Face Cosmetic, Date palm, therapy.

ABSTRACT

Background: It has been proven that adding date kernel extract to cosmetics as therapeutic supplements, due to antioxidant and antiviral content, is safe and effective in rejuvenating patients' faces .**Objectives:** This study aims to evaluate the effectiveness and safety of a formulated cosmetic gel **(CG), and** date palm kernel methanol extract (DPKME) alone and in combination with FCG@DPKME in treating and enhancing facial skin quality in women using a protocol developed by local cosmetic experts.**Patients/Methods**: In this study, Herpes virus was isolated and identified using biological and molecular methods from clinical specimens collected from pus and fluids from gynecological patients with edema, erythematous boils, and vesicles. Using Gas chromatography-mass spectrometry (GC–MS) analysis of DPKME. A combination formula Face cosmetic gel **(CG),** and date palm kernel methanol extract (FCG@ DPKME) prepared and applied externally directly to the skin surface of female patients weekly and continued for 14 weeks. Evaluations included therapeutic assessments of edema, erythematous boils, and biophysical assessments covering the four emerging perceptual categories (EPCs) of skin quality and a subjective assessment using the Global Aesthetic Improvement Scale.**Results:** The isolated virus was identified as Herpes Simplex Virus-1(HSV -1)using PCR by specific primers with 99.25 % similarity, related to HSV strains recorded PLAST in Gene Bank . HSV -1 treated with Face cosmetic gel (CG), date palm ***(phoenix dactylifera L.)*** kernel methanol extract CG@DPKME in Vero cell line in vitro. CG@DPKME showed longevity and reduced virus titer by 95.5% compared to 90.5% with date Palm kernel methanol extract (DPKME) alone and 45.7% with the basic cosmetic gel (CG). The DKME remained effective between 4 and over 12 months after being preserved in the cosmetic formula In Vitro. The DPKME@CG remained effective between 4 and over 12 months after being preserved in the cosmetic formula In Vitro. Significant improvements were also observed in skin glow (skin luster, skin hydration and elasticity, skin surface evenness, improved skin porosity, transepidermal water loss, and treatment of edema, erythematous boils, and vesicles). At week 12, most subjects and investigators reported an improvement in overall appearance with treatment, with a 100% improvement rate. No serious adverse reactions were observed.**Conclusions:** Our study provides real-world insights into the efficacy and safety of date kernel extract in improving facial skin quality in women, evaluated through objective and subjective assessments.

INTRODUCTION

The most common Skin illnesses are caused by a diversity of pathogens, like bacteria, viruses and fungal species. Viruses often involved in skin infections include Herpes viruses, in addition to bacteria that cause diseases such as cellulitis, erythema, redness, herpes, folliculitis, and boils (Nesy, and Mathew, 2014).

Medicinal plants are utilized to manage skin illnesses, and the biological efficacy of these plants is attributed to the existence of various secondary metabolites like phenols and flavonoids (Grierson, *et al.,* 2014 and Otang*, et al.,* 2012). In addition, medicinal plants act as a source of bioactive substances of pharmacological interest to humans (Otang, *et al.,* 2012 and Atanasov, *et al.,* 2015). The importance of scientific testing of the efficiency of plants in biological tests (e.g., antimicrobial, antioxidant, anti-inflammatory activities) associated with skin illnesses and evaluating their safety was well highlighted (Mabona and Van vuuren, 2013). To date, reports on the biological activities, safety and phytochemistry of several medicinal plants utilized in folk medicine were not discovered (Lall, and Kishore, 2014). They are both well mentioned as folk remedies for skin illnesses. These plants are recognized for their varied biological activities, involving antimicrobial, antioxidant, anti-toxic and anti-inflammatory activities (Bozorgi, *et al.,* 2017 and Mpofu, *et al.,* 2014), in addition to their rich phytochemical pools (Sevil, *et al.,* 2015). Although the varied biological activities of these medicinal plants, their influences against microbes involved in prevalent skin illnesses aren't well reported. Therefore, the present research investigated the pharmacological activities (antimicrobial and antioxidant) and phytochemical composition in addition to the safety of 4 plants utilized in the management of skin illnesses.

The quality of cosmetic gel has a profound effect on attractiveness, motivating many individuals to seek aesthetic procedures to improve their appearance and cure them from virus and bacterial infections. (Goldie *et al.,* 2021 and Park., *et al.,* (2022). A panel of global beauty experts identified four traits, known as emergent perceptual categories (EPCs). which describes good skin quality across different ethnicities: (1) skin radiance, (2) skin firmness, (3) skin surface, and (4) uniform skin tone [1]. The perception of good skin quality is shaped by several traits or EPCs, which are interconnected and can influence each other (Rutnumnoi, *et al.,* 2025).

Herpes simplex virus (HSV) is a common skin infection that can cause painful sores or ulcers. It is spread primarily by skin-to-skin contact. It is treatable but not curable. In 2020 (latest available estimates), 3.8 billion people below the age of fifty, or 64% of the world's population, were influenced by herpes simplex virus form. Most HSV-1 infections are acquired during childhood to cause oral herpes, although the largest number of new infections occurs among adolescents and young adults. An estimated 205 million people aged 15-49 years (5.3%) suffered from infection (WHO, 2024).

The date palm is a succulent plant that has been utilized for centuries for its medicinal properties, involving it's antimicrobial activity. Palm kernel gel comprises a diversity of bioactive substances like saponins, anthraquinones, polysaccharides, and antioxidant that were demonstrated to have antiviral, antibacterial, anti-inflammatory & wound-healing properties, making it a valuable aid in treating skin infections (Kumar, *et al.,* 2019 and Abo-elmaaty, *et al.,* 2016).

Date palm kernel extract (DPKME) represents promising alternative therapies that can enhance antibacterial and antiviral activities and enhance clinical results. Their

combined utilization can result in more effective treatment of skin infections, especially those resulted from herpes virus and multidrug-resistant pathogens and cure these infections (Sánchez, *et al.,* 2020).

The current aimed to objectively evaluate the biophysical efficacy of GC@DPKME for all four EPCs for skin quality using a protocol developed by local cosmetic experts to improve cosmetic gel quality. To reflect clinical trials and provide practical insights into treatment outcomes.

MATERIALS AND METHODS

Collection and Preparation of Clinical Specimens:

One hundred clinical specimens collected and approved through the Research Ethics Committee at Ain Shams University, Agriculture Sector Committee. Clinical specimens 50 female and 50 male patients have been gathered from erythematic furuncles, edema, and vesicles which formed one hundred clinical swabs specimens pus & fluid at the Last stage of infection. In dermatology hospital in Qalyubia governorate. The pus and fluid edema gave positive HSV antigens (oral herpes) via forty milliliters of ten percent beef extract has been added to 25 ml and stirred for 30 minutes. at room temperature. The mixture has been centrifuged at 12000 rpm for fifteen minutes under cooling, the pellet has been discarded, and the supernatant has been then concentrated via organic flocculation technique according to Katzenelson, *et al.* (1976). The pus and fluid edema were treated a 1/10 dilution with 1:2 of trypsin (Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.) per ml at 37°C for 15 min.

Source and Preparation of Date Palm Kernels Methanol Extract (DPKME):

The five Kg of Siwi date palm kernels (DPK) P. *dactyliferapits* was brought from the Central Lab. for Research and Development of Date Palm, ARC, GIZA, Egypt. It was washed with distilled water, and air dried, DPK ground into fine powder before Cutting Mill (SM 400, RETSCH, Germany). being conserved at -20ºC according to **López *et al.* (2022).**

The DPK Methanolic extract (DPKME) was prepared by weighting 100 g of fine powder of DPK dissolved in 500 milliliters absolute Methanol and mixed well, subsequently left for twenty-four hours at ambient room temperature. Next, the mixture has been filtered through filter paper (Whatman No.1) and concentrated through rotary vacuum evaporator at forty degrees Celsius subsequently left to dry. Subsequently gathered and stored in dark-dry place (Bouhlali *et al.,* 2015) Methanolic extract of Date palm kernels

Analysis of DPKME Active Compounds by GC-Mass:

The chemical composition of DPKME has been conducted utilizing Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, United States of America) with a direct capillary column TG–5MS (30 meters x 0.25 millimeters x 0.25 micrometers film thickness). The column oven temperature was initially held at fifty degrees Celsius and then elevated by five degrees Celsius per minute to 230 degrees Celsius held for two minutes. and then elevated to the final temperature 290 degrees Celsius by thirty degrees Celsius per minute then held for two minutes. The injector and MS transfer line temperatures have been maintained at 250, 260 degrees Celsius correspondingly; Carrier gas used was Helium at a continuous flow rate of one milliliter per minute. The solvent delay was three minutes, and diluted samples of one micrometer have been injected automatically utilizing Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra have been gathered at seventy eV ionization voltages over the range of m/z 40–1000 in full scan mode. The temperature of ion source has been established at 200 degrees Celsius. The elements have been identified through comparison of their retention times and mass spectra with those of Wiley 09 and Nist 11 mass spectral database (Dawwam *et*

al., 2022) at The Regional Center for Mycology and Biotechnology Laboratory, Al-Azhar University, Cairo, Egypt.

Preparation of FCG @ DPKME formula:

Facial cosmetic gel (FCG) was prepared by mixing the following ingredients: four percent stearyl alcohol, eight percent stearic acid, five percent glycerin, three percent Ceto stearyl alcohol, three percent isopropyl myristate, five percent paraffin oil, 0.5 percent active ingredient, five percent Tween 20, seven percent perfume, and one percent of methyl paraben. Sodium hydroxide has been added dropwise to this mixture along with 0.1-0.5 percent Date palm kernels methanol extract (DPKME) using a vortex mixer **(**Carli, 2020). Separately, Carbopol was completely dissolved in 100 ml of distilled water, & subsequently sodium hydroxide has been added dropwise with stirring to create the gel.

Preparation of Established Cell Lines:

Vero cell cultures: The selected Vero cell lines, have been attained from American Type Culture Collection (ATCC) continuous cell line established via (Yasumura and kawakita, 1963). Vero cells continue passaging to achieve high viral titters (104 cells) 0.1 mL of and freezed -70oC used to inoculate a fresh vial of Vero. Cell count using Hemocytometer was performed using trypan blue (Sigma Aldrich-USA) vital stain as mentioned under the enumeration of cells was performed according to Schmidt, (1970).

Microtitre Plate Cell Culture:

The Vero cultures cell culture (104 cells) was diluted (1:10 dilution) using growth medium to contain cells. For seeding 12 well microtiter plate / 0.1 mL of (104 cells / well / 0.1 mL Dulbecco's Modified Eagle's Medium (DMEM) with Hanks's salt base, supplemented with ten percent fetal calf serum and fifty (micrograms per milliliter) gentamycin antibiotic solution) and leave for five minutes at room temperature then incubated at 370C for 24 hrs. in a humidified incubator with five percent CO2 to permit attachment developed complete monolayer sheet of cell. Microtiter plate Vero cell culture was used to determinate cytotoxicity, virus titration and antivirus activity.

Detection, Isolation and Identification of Herpes virus (HSV):

Detection of HSV:

First Identification of viral antigen, Immunochromatography assay (Latex agglutinin lateral – flow immunoassays) was used for the recognition HSV antigens in concentrated pus and fluid using Rapid Card Insta Test California a1302 USA, 176666-1 according to Afifi *et al.* (2009). Three drops (120-150 ul) were delivered of diluted sample to the sample well. The result was recorded between 5-10 min.

Isolation and Propagation of HSV:

HSV was isolated and propagated on Vero cell line culture according to Afshar (1992). Confluent monolayer cultures of Vero cells (16 x 125 millimeters culture bottle) have been rinsed one time with phosphate-buffered saline PBS) pH 7.2), managed with phosphate-buffered saline supplemented with 2.5 / tg 2 x crystalline trypsin/ml and inoculated with 0.1 milliliter explained pus and fluid edema for ten min at thirty-seven degrees Celsius. After one-hour adsorption, cell cultures have been washed once with phosphate-buffered saline, fed with one milliliter mM and incubated at thirty-five degrees Celsius. When the cells demonstrated fifty to eighty percent cytopathic alteration, the culture bottles have been frozen and thawed rapidly 3 times, then centrifuged at 4000 rpm for ten minutes and supernatants have been inoculated into fresh cell cultures as mentioned above (WHO, 2009**).** The total multiplicated HSV considered as virus stock that was divided into 1ml aliquots into sterile screw capped plastic vials and stored at -70°C. Maintenance medium was dispensed to infected plates and incubated at thirty-seven degrees Celsius for two days, with daily microscopic examination for detecting the CPE of test virus. At the end of experiment duration, 2 days the tissue

culture infective dose (50) for each virus model was determined according to Reed,*et.al.,* (1938)

Determination of HSV by Plaque Forming Unit (PFU) Assay:

The guideline was previously defined (Tache, *et.al.,*1995). The plaques have been numbered following three to five days. The virus titter has been measured as a plaque-forming unit per milliliter (PFU / milliliter). Confluent monolayers of Vero cells have been prepared in 12 well plates (Nunc). Serial ten-fold dilutions have been prepared of virus in chilled maintenance medium (MEM-E with one percent serum). Culture medium has been eliminated & 0.2 milliliter of (10^6PFU/ milliliter) virus inoculum has been added, starting from the greatest dilution. The plates have been incubated at thirty-seven degrees Celsius for seventy-two hours with intermitted added, starting the plate. The inoculum has been eliminated, preferably with a pipette & subsequently 1.5 milliliters of agarose overlay medium (growth medium have been added with one percent agarose & two percent FCS). It was left at room temperature for ten minutes & thereafter incubated at thirty-seven degrees Celsius. The monolayer has been investigated daily starting from 2nd day of incubation. Once the plaques had formed, typically by the 4th day after inoculation, the numbers of plaques have been counted at every dilution, the agarose overlay has been eliminated & the monolayer was gently rinsed with phosphate-buffered saline. The plate has been stained with 0.1 percent crystal violet solution & the plaques have been counted again. The virus titter has been measured as PFU per milliliter as described via Tache, *et al.* (1995).

Molecular Identification of HSV:

Extraction of Total DNA:

The total DNA from normal and infected Vero cells was extracted using the Gene JETTM DNA purification kit (Fermentas, USA) following the manufacturer's instructions for molecular detection. For the home-brew molecular assay, a DNA blood kit (Qiagen, Hilden, Germany) which needed 200 microliters of sample has been utilized based on the manufacturer's instructions.

Primer Design:

This set of primers was newly utilized for identification of herpes simplex virus encephalitis through nested PCR (Powell, *et al.,*1990). The primer sequences and characteristics (Kessler,*et al.,*2000) (Table 1).

Table 1: Primer sequences pairs designed specifically the glycoprotein D region of the HSV genome.

Target gene	Primer Sequence 5′–3′	MT (°C)	Amplified positions		
			HSV-1	HSV-2	Product
DNA glycoprotein D gene	F (5′-TGCTCCTACAACAAGTC -3	70.1	138878–138897	141475–141494	142
	R (5′-TCTCCGTCCAGTCGTTTA TCTTC -3')	57.3	138997-139019	141594–141616	

Amplification of Glycoprotein D Region by PCR:

The PCR has been conducted in a fifty-microliters reaction mixture comprising twenty-five microliters of Taq polymerase chain reaction master mix solution (Qiagen), thirteen microliters of double-distilled, DNase-free water, one μ M concentration of every primer, and ten microliters of the extracted sample. Polymerase chain reaction has been conducted using a PE 9600 thermocycler (Perkinelmer cetus, Branchburg, N.J.). 35 cycles were conducted, comprising denaturation at ninety-five degrees Celsius for thirty seconds (five minutes throughout the first cycle), annealing at fifty-five degrees

Celsius for thirty seconds, and extension at seventy-two degrees Celsius for thirty seconds. Following the final cycle, the tubes were incubated for another five minutes at seventy-two degrees Celsius and thereafter cooled to five degrees Celsius. Nested amplification has been performed using a five-microliters aliquot from the first run, along with twenty-five microliters of Taq polymerase chain reaction master mix solution, eighteen microliters of double-distilled, DNase-free water, and a one μ M concentration of every nested primer, following the same cycle protocol as previously reported.

Detection of glycoprotein D region DNA amplification:

Ten microliters of each PCR product have been examined on one percent agarose (Prona, Spain) gel comprising ethidium bromide (Sigma, USA). NCDV (G6P) strain was used as positive control for Rotavirus. Nuclease free water was used as negative control. Subsequently, PCR products (five microliters) have been separated on one percent ethidium bromide-stained agarose gel electrophoresis at 120 V for twenty minutes. DNA Marker (1-kbp) has been utilized as standard and the amplicons have been visualized utilizing ultraviolet light transilluminator (Spectroline) **(**Sambrook, *et al.,*2001). Gel Analysis have been photographed scanned, examined utilizing Gel Doc VILBER LOURMAT system.

Virus-informatic Analysis:

Determination of Glycoprotein D Region Sequencing:

PCR fragment product for HSVQ -1 isolate was purified utilizing Gene JET PCR purification kit (Fermentas). The purified PCR was direct sequenced by the dideoxy chain termination method, using a DNA sequencer (Macrogen Inc., Korea ABI 3730XL DNA analyzer). DNA sequences of HSV isolate were aligned utilizing Bio Edit software version 7 (www. Mbio-NCUs. Edu/bio. Edit). The nucleotide sequence of HSV isolate compared with other accessions of HSV isolates accessible in the NCBI information base utilizing BIASI- algorithm to recognize closely associated sequences (http/WWW.NCbI.Nih.Gov).

Dendrograms have been constructed through utilizing unweighted pair Group method with Arithmetic (UPGMA).

Examination of Cytopathic Effects (CPE) of HSV on Vero Cell Line:

The plates of inoculated Vero cell have been examined below an inverted microscope (HUND-Germany) following three to six days. Effect of viral infection was considered based on the development of CPE. When approx. CC50 % of cells demonstrated cytopathic alteration, coverslips have been eliminated, washed with phosphate-buffered saline, fixed with methanol, stained with May-Grfinwald-Giemsa stain and investigated for inclusion bodies according to **Badawi, *et al.*, (2016).**

Evaluation of DPKME as Antiviral Activity:

Cytotoxicity and Maximum Non-Toxic Concentration:

DPKME anti-proliferative activity was determined utilizing MTT assay on Vero cells have been grown as monolayer. The cytotoxicity has been evaluated utilizing MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and examined by microscopic observation as described by Subhashini, *et al.* (2013). Different concentrations of DPKME (100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 (micrograms per milliliter) have been added to the cell monolayer. Triplicate wells have been prepared for every concentration except for another 3 wells without extract as negative control. The plates have been incubated for 48h into CO2 incubator at thirty-seven degrees Celsius and five percent CO2 and examined under inverted microscope to observe cytopathic effect of DPKME on treated Vero cells cell. The monolayer cells plate has been rinsed twice with phosphate buffer saline. MTT solution (50 microliters of five milligrams per milliliter stock solution, Bio Basic Canada INC) has been added to every

well, & incubated at thirty-seven degrees Celsius for four hours, followed by medium discarded. In each well, 200 microliters of acidified isopropanol (0.073 milliliter HCl / fifty milliliter isopropanol) have been utilized to dissolve the produced formazan crystals. The reduction of MTT into formazan (purple needle color) through dehydrogenase activity in mitochondria of viable cells. 100 (microliters) of dimethyl sulfoxide has been added to every well to solubilize the purple crystals of formazan. Absorbance has been determined at (570nm) with microplate reader. The percentage of cell survival has been estimated via the following equation.

$$\text{Survival rate \%} = \frac{A\ \text{sample} - A_b}{A_c - A_b} \times 100$$

A_c=Negative control A_b=Blank

The maximum nontoxic concentration (MNTC) has been determined. The experiment has been repeated three times for each cell line. Cytopathic effect inhibition assay using MTT:

$$\text{Cytotoxicity(\%)} = \frac{\text{Abs.of untreated cells - Abs.of treated cells}}{\text{Abs.of untreated cells}} \text{X100}$$

The absorbance of the formazan solution has been determined at λ max 540 nanometers, with 620 nanometers as a reference wavelength utilizing a spectrophotometer. Optical density (OD) has been directly related to cell amount. The decreased the absorbance of managed cells by fifty percent (CC50) value has been described as the concentration in comparison with unmanaged cells and this has been determined through regression analysis utilizing the plot of cytotoxicity (%) against samples concentration.

Determination of DPKME Antiviral Activity.

The monolayer cells have been plated (10^4 cells/well) in twelve-well tissue culture plate and incubated for twenty-four hours at thirty-seven degrees Celsius in a humidified incubator with five percent CO2. After 24h of incubation, the medium has been substituted with one hundred microliters of MEM comprising 50 µL of MNTC DPKME and fifty microliters of 100 TCID50/50 microliters of HSV were added in triplicate & incubated for three days. Controls comprised infected unmanaged and untreated non infected (cellular control). The quantification activity of DPKME showed cell viability & indirectly cell extract protection against the virus. All experiments have been conducted in triplicate. The protection percentage has been estimated utilizing the following formula:

$$\text{Protection (\%)} = \frac{\text{Abs. treated virus - Abs.of virus control}}{\text{Abs, of cell control - Abs.of cell virus infected}} \text{X100}$$

Determination of DPKME Cytotoxicity Activity:

The cell proliferation and cytotoxicity of Vero with DPKME were measured according to the method of Mosmann (1983). To assess the half maximal cytotoxic concentration (CC50), DPKME was prepared in 10 % DMSO in ddH2O and diluted further to the working solutions with DMEM. Cytotoxic activity was tested against Vero-E6 cells by using the 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method with minor modifications. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×105 cells/ml) and incubated for 24 h at 37 °C in 5%CO2. After 24 h, cells were treated with various concentrations of DPKME in triplicates. The supernatant was discarded, and cell monolayers were washed with 1x phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 h followed by medium aspiration. In each well, the

formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 ml HCL in 50 ml isopropanol). Absorbance of formazan solutions were measured at λ max 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation. The plot of % cytotoxicity DPKME concentration has been utilized to estimate the concentration, which showed fifty percent cytotoxicity (CC50).

$$\%\text{=Cytotoxicity} = \frac{\text{Absrbance of unteated cell - absorbance of treated cell}}{\text{Absrbance of unteated cell}} \text{ X 100}$$

Antiviral activity Assay:

The relation between percentage viability cell line and DPKME concentrations has been examined to get the Inhibitory Concentration 50 (IC50) (dose of the medication which decreases survival to fifty percent) utilizing SigmaPlot 12.0 software (Alahdal, *et.al.,* 2018). In 96-well tissue culture plates, 2.4×104 Vero-E6 cells have been distributed in every well & incubated overnight at a humidified thirty-seven degrees Celsius incubator under five percent CO2 condition. The cell monolayers have been then rinsed once with 1x phosphate buffer saline and exposed to HSV-1 adsorption for one hour at room temperature (RT). The cell monolayers have been additional overlaid with 100µl of DMEM comprising differing concentrations of the test compounds. After incubating thirty-seven degrees Celsius in five percent CO2 incubator for seventy-two hours, the cells have been fixed with one hundred micrometers of four percent paraformaldehyde for twenty minutes and stained with 0.1 percent crystal violet in distilled water for fifteen minutes at room temperature. The crystal violet dye has been subsequently dissolved utilizing one hundred micrometers absolute methanol per well & the optical density of the color is determined at 570 nanometers utilizing Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC_{50} compound is needed to decrease the virus-induced cytopathic effects by fifty percent, relative to the virus control.

Preparation of Facial Cosmetic gel and DPKME Formula:

The relation between percentage viability cell line and DPKME concentrations has been examined to get the Inhibitory Concentration 50 (IC50) (dose of the medication which decreases survival to fifty percent) utilizing SigmaPlot 12.0 software (Alahdal, *et.al.,* 2018). In 96-well tissue culture plates, 2.4×104 Vero-E6 cells have been distributed in every well & incubated overnight at a humidified thirty-seven degrees Celsius incubator under five percent CO2 condition. The cell monolayers have been then rinsed once with 1x phosphate buffer saline and exposed to HSV-1 adsorption for one hour at room temperature (RT). The cell monolayers have been additional overlaid with 100µl of DMEM comprising differing concentrations of the test compounds. After incubating thirty-seven degrees Celsius in five percent CO2 incubator for seventy-two hours, the cells have been fixed with one hundred micrometers of four percent paraformaldehyde for twenty minutes and stained with 0.1 percent crystal violet in distilled water for fifteen minutes at room temperature. The crystal violet dye has been subsequently dissolved utilizing one hundred micrometers absolute methanol per well & the optical density of the color is determined at 570 nanometers utilizing Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC_{50} compound is needed to decrease the virus-induced cytopathic effects by fifty percent, relative to the virus control.

Determination of FCG@DPKME Antiviral Activity:

Vero cells have been grown as monolayer in media supplemented with ten percent inactivated fetal bovine serum. The monolayers of (10,000) cells have been plated (104 cells/well) in 96-well tissue culture plate and incubated for twenty-four hours at thirty-seven degrees Celsius in a humidified incubator with five percent CO2. After 24h of incubation, the medium has been substituted with one hundred micrometers of MEM comprising 50 µL MNTC of FCG@DPKME and fifty micrometers of 100 TCID50/50 micrometers of HSV was added in triplicate & incubated for three days. Controls comprised unmanaged infected (virus control at 100TCID50/50 micrometers), managed non infected (DPKME), and unmanaged non infected (cellular control). The cytopathic effect was detected following 72h & the extracts with antiviral activity were determinate. For quantification of this activity. This assay permits the quantification of cell viability & indirectly permits quantification of cell extract protection against the virus. All experiments have been conducted in triplicate. The protection percentage has been estimated utilizing the following formula.

$$\text{Protection (\%)} = \frac{\text{Abs. treated virus - Abs.of virus control}}{\text{Abs, of cell control - Abs.of cell virus infected}} \text{ X100}$$

Assessments of FCG@DPKME:

Facial skin improvement assessment included objective biophysical assessments of the four EPC components of skin quality: (i) Facial skin type (ii) Skin color tone (iii) Skin texture (iv) Skin moisture) And other characteristics before use and after 15 and 30 days of use Biophysical markers of quality were measured using corresponding devices (Skin Gloss Meter (Delfin Technologies Ltd., Finland) Moisture meter D (Delfin Technologies Ltd., Finland) In line with the four standards defined in the Global Consensus on Skin Quality (Goldie, *et al.* (2021) **.**

Statistical Analysis:

The experiments have been performed in 3 separate tests. Antibacterial activity information was statistically assessed utilizing Statistical Package for the Social Sciences for Windows 16.0. Variances between means have been tested at a significance level of P-value below 0.05. Outcomes have been regarded statistically significant if P-value below 0.05, with distinct superscripts (a, b) demonstrating significant variances (P-value below 0.05).

RESULTS

Human Herpes Virus (HSV) Identification:

Herpes Virus Prevalence:

HSV infection was identified depending on distanced clinical symptoms Acute inflammation and tissue skin damage required hospitalization collected from woman and man at different hospitals belong Qalyubia and Cairo governorates (Table,2). As well as it has been serologically observed in concentrated clinical pus and fluid of using ILFST test. Principally in Females for this study who attending hospitals HSV antigen was found in 30 out of 80 specimens. Whereas HSV has been found in man 7 have been observed out of 20 clinical samples (Table 2).

Table 2: Prevalence HSV in concentrated clinical pus and fluid in Cairo and Qalyubia based on distance clinical symptoms and ILFST test.

Location	Clinical Specimens	Gender	Clinical symptoms	Positive Ag HSV
Qalyubia (n=50)	pus and fluid (n=50)	Female (n=40)	ASIE, VP, ITSD, HB, EF	18
		Male (n=10)	EF, VP, VF, HB	4
Cairo (n=50)	pus and fluid (n=50)	Female (n=40)	ASIE, VP, ITSD, HB, EF	12
		Male (n=10)	EF,, VP , VF , HB	3

Significant differences were calculated using a one-way ANOVA according to the least significant difference (LSD) method at a p _ 0.05 level of probability with the GraphPad Prism software package.
Acute severe infection edema (ASIE), erythematic furuncles (EF) , vesicles that produced pus (VP)and fluid (VF) , inflammation and tissue skin damage (ITSD) , resulting in the characteristic herpes blisters(HB) , Ag = Antigen was detected by ILFST

Biological Identification:

Four ILFST positive HSV clinical specimens namely (HSVQ1 & HSVQ2) from Qalubia and (HSVC1 & HSVC2) from Cairo distanced acute severe infection were isolated using Vero cell. The infected Vero cells demonstrated cytopathic effects (Figure 2) and reduced cell viability (table,3) at 3 to 4 days incubation by inverted microscope.

Cytopathic Effect (CPE) of HSV:

Assayed on Vero cells using MTT. HSV infection showed plaques and cytoplasm granulation, inclusion bodies and rounding and swelling cells, destroyed, elongated, multinucleated (giant cells) and intra cytoplasmic inclusions with different sizes and shapes compared with healthy normal spindle cells. Then macrocytes of infected cells were fractal appearance and have lytic degeneration. The second form of cytopathic effect involved the formation of a multinucleated giant cell after five days (Figs. 1&2).

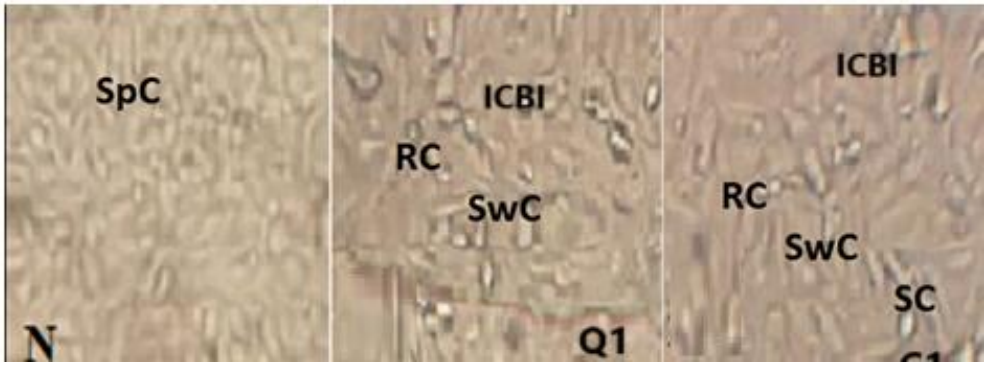

Fig.1: Vero cell line (N) normal cell and (D) incubated HSV infected cells for 6 days (Q1 & C1) showing cytopathic effects Spindle cell (SpC), Swelling cell (Swc), Round cell (RC), Spinal cell (SC) , intra cytoplasmic inclusions (ICBI) Giant cell (Gc) and destroyed cell (dC) survival cells.

Cell Viability:

The count of died cells was Log 2.03 ,1.69 ,1.96 & 1.62 % and survival cells 3.27, 3.29 ,3.28 & 3.29 out of staining cell = Log 3.33 of Vero suspension cell for HSVQ1, HSVQ2, HSVC1 and HSVC2 respectively (Table 3).

Table 3: Count of survival and died of Vero cells infected with HSVQ1, HSVQ2 , HSVC1 & HSVC2 HRV isolates.

HSV isolates		HSVQ1	HSVQ2	HSVC1	HSVC2
Log Vero Cell count	Died	4.25 a	3.59 b	3.48 a	3.65 b
	Survival	1.43 c	1.65 a	1.95 b	1.64a
Significant differences were calculated at the least significant difference (LSD) method at a p _ 0.05 level of probability. The mean values of each column with the same letter do not differ significantly.					

The initial Vero cells count (Log 3.33) cells.

HRV Titer:

The HSVQ1, HSVQ2, HSVC1 and HSVC2 titer have been calculated as plaque forming units (PFU/ml) on monolayer Vero cell line. The titers of four HSV isolates were different 1.2x106, 3.0x106, 4.2x106 and 7.5x105 plaque forming units per milliliter respectively following 4 days post infection (Table, 2 and Fig.2).

Table 4: Titration of Human HSV as plaque forming units (PFU\ml) on of Vero monolayer cell line.

HSV isolates	Qalubia governorate		Cairo governorate	
	HSVQ1	HSRVQ2	HSVC1	HSVC2
Titer PFU/ ml	$6x10^5$	$4x10^5$	$5x10^5$	$3x10^5$
Significant differences were calculated at the least significant difference (LSD) method at a p _ 0.05 level of probability. The mean values of each column with the same letter do not differ significantly.				

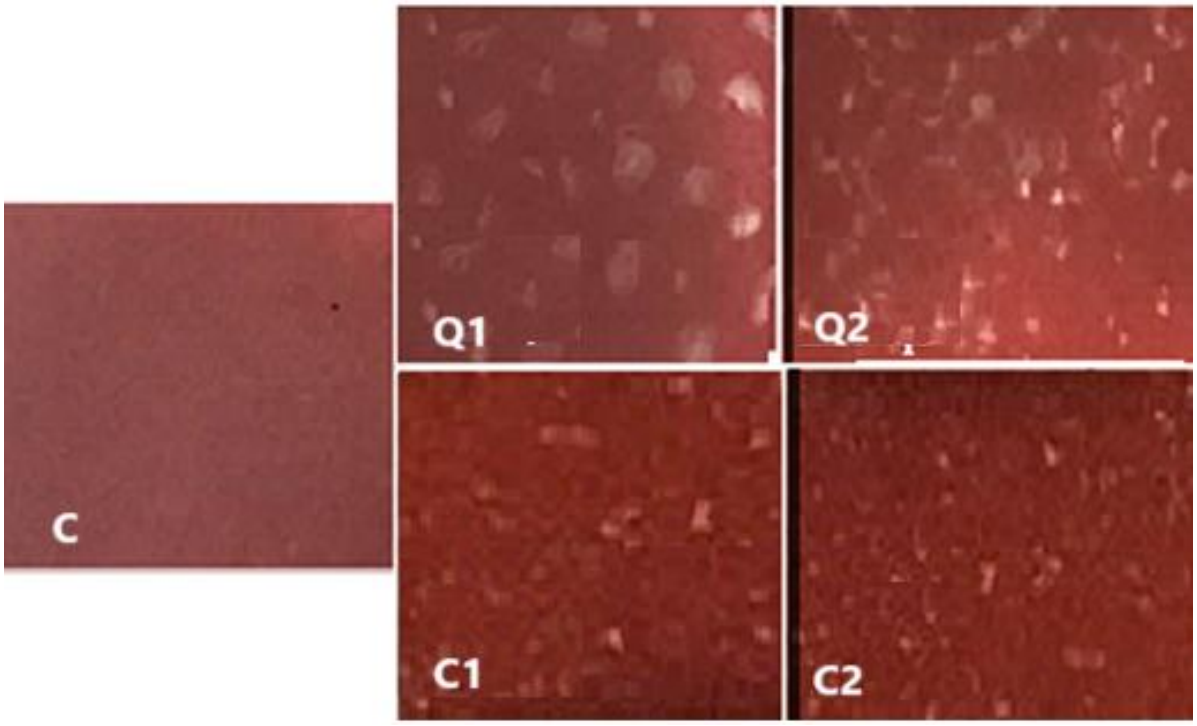

Fig.2: Photo monolayer Vero cell lines infected with four, HSVQ1, HSVQ2 , HSVC1 & HSVC2 virus isolates and normal cell (C) at 72 h incubation investigated by invert microscope showing different morphology plaques compared with normal cell non formed plaques.

Molecular Identification:

UV Spectra Characters of HSV Isolates:

The UV minimum (235, 232, 234 & 229) and maximum (260, 259, 260 & 260 OD) of four HSVQ1, HSVQ2, HSVC1 & HSVC2 virus isolates respectively. The integrity and amount of the purified total DNA extracted from infected Vero cells with four HSV isolates confirmed by agarose gel electrophoresis and UV spectrophotometer. The DNA yields were 98, 76, 82 and 76 µg /µL and the purity was measured by an A260/280 absorbance with ratio (2.09, 2.11, 2.22 & 1.81) for four isolates respectively (Table,5).

Table 5: UV spectrum characters total DNA, Purity and yield of HSV isolates**.**

Isolates	UV spectrum characters						Yield (ng/uL)
	Minimum (OD)	Maximum (OD)	260(nm)	280(nm)	260/280	280/260	
HSVQ1	235	260	0.575	0.275	2.09	0.478	98
HSVQ2	232	259	0.525	0.248	2.11	0.472	76
HSVC1	234	260	0.499	0.225	2.22	0.440	82
HSVC2	229	260	0.475	0.276	1.81	0.581	76

Amplification of Glycoprotein D region DNA of the HSV Genome:

DNA - glycoprotein D gene of four HSV isolates have been amplified through PCR reaction mixture and specific primer sets. The PCR product has been confirmed utilizing 1.5 percent agarose gel electrophoresis with the expected size calculated ≈ 400 bp related to leader molecular DNA (Fig. 3).

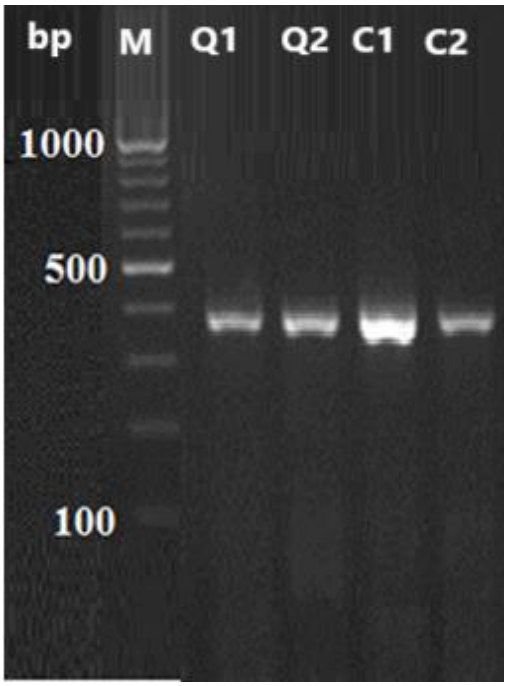

Fig. 3: Agarose gel 1.5 % electrophoresis showed PCR products with expected size ≈ 400 bp from Vero cell infected with HSV using a glycoprotein D region primer (lanes Q1 (HSVQ1), Q2 (HSVQ2) , C1(HSVC1) & C2 (HSVC2) virus isolates. M = DNA leader molecular marker.

Virus Informatic Analysis of Glycoprotein D Region of HSVQ1 Isolate:

The partial nucleotide sequence (Fig. 4) of glycoprotein D region gene for HSVQ-1 isolate has been performed to determine the relationship with additional recommended HSV isolates registered in GenBank.

HSV-1(HSVQ1 isolate) From 1 to 399:

Atgagccgagacgccaggcacgcggccctgcgccgacgtttggccgagacgcacctccgggccgaggtgtacag
ggaccagaccctgcagctacaccgggagggcgtgagtacacaagatcctaggtttgttggcgcctttatggccgcca
aggcggcccacttggaattggaggcgcggctaaagtcccgcgcgcgcttagagatgatgcgacagcgagcgacct
gtgttaaaattcgcgtggaagagcaagcggcgcgacgtgattttctaaccgcacaccgacggtacctcgatcccgcgc
ttagcgagcgcctggacgcggcggacgatcgtcttgcggaccaggaggagcagctcgcagaggccgcggcgaac
gc gtctctgtgggaggacggcga

Fig.4: Partial nucleotide sequence 399 bp fragment of glycoprotein D region DNA gene for HSVQ1 isolate.

Multiple Alignment of Nucleotide Sequence:

The sequencing has been performed from the forward direction at Macrogen3730XL6-1518-009, Korea. The sequence fragment of C-terminal region of glycoprotein D region HSVQ1 isolate has been performed to determine the relationship with additional registered HSV strains in GenBank (Fig. ,5). The multiple alignment of partial nucleotide sequence for vp6 gene HSV isolate submission ≠2691714 with 18 HSV isolates documented in the GenBank utilizing DNAMAN and MEGA.4 programmers (Wisconsin, Madison, United States of America). Multiple sequence alignment (MSA) has shown Max Score or total Score varied 983 to 977 total score, varied to 97.55, Quarry Cover 100%, E. value 0.0 percentage identity ranged 97.73 to 97.55.

Phylogenetic Tree:

Based on multiple alignment of partial nucleotide sequence for glycoprotein D region DNA gene HSV of HSVQ1 isolate and MSA analysis, the phylogenetic tree has been conducted and demonstrated two clusters in which HSV of HSVQ1 isolate with 100 HSV isolates recorded on GenBank. The similar percentage was 99.55% (Figure.,6). The genetic distance was 0.001 of HSVQ1 isolate Query_7241739 related to 100 HSV isolates recorded on GenBank based on multiple alignment of nucleotide sequence GenBank (Fig. 5).

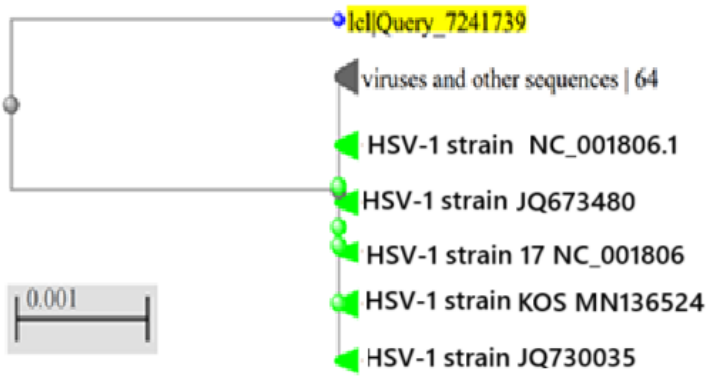

Fig. 5: Phylogenetic tree of glycoprotein D region DNA gene nucleotide sequence homology of isolated HSVQ1 and 100 HSV isolates published in GenBank. Numbers represent bootstrap percentage values based on 1000 replicates.

Antiviral activity of DPKME:

Active Gradient Compounds of DPKME:

The phytochemical composition of DPKME was determined by GC-mass analysis. It confirmed that 37 peaks at different retention times consist of 37 active compounds, consisting of essential and effective components that exhibit numerous bioactive activities. These components as Flavonoids. (Rutin, Querestin, Luteolin, Kampfero, Catechin) and phenols (Syringic, Caffeic, Ferulic and Epicatechin) (Table 6; Fig.6) derivatives the peaks appear at retention time 12.0 and finished at 40.6. These components showed antiviral, anti-inflammatory, antioxidant biological activities compound the graph show condensed peak of compounds precipitated, results estimated by determining the Molecular weight and molecular formula through PubChem and PubMed website (Table 6 and Fig.6).

Table 6: The abundance of active compounds of DPKME determined by GC-mass analysis at different retention times.

Classification	RT#	Compound and IUPAC Name	Concentration µg/gm	MW (g/mol)	Molecular Formula	Biological activity
Flavonoids	5	Rutin, vitamin C (2(3,4dihydroxyphenyl)-5)	4.22	610.5	$C_{27}H_{30}O_{16}$	Food Additives Drugs Antiviral Antitumor anti-inflammatory antioxidant Cosmetics Hair, Skin conditioning
	7	Querestin (3,3′,4′,5,7-Pentahydroxyflavone)	5.12	302.236	$C_{15}H_{10}O_7$	
	9	Luteolin (3',4',5,7-Tetrahydroxyflavone)	3.78	286.24	$C_{15}H_{10}O_6$	
	10	Kaempferol (3,4′,5,7-tetrahydroxyflavone)	10.36	286.23	$C_{15}H_{10}O_6$	
	12	Catechin (2-(3,4-dihydroxyphenyl)-3)	8.33	290.26	C15H14O6	
	12	Epicatechin (2-(3,4-dihydroxyphenyl)-3)	13.44	290.26	C15H14O6	
Phenols	5	Syringic acid (4-Hydroxy-3,5-dimethoxybenzoic acid)	7.25	198.174	$C_9H_{10}O_5$	Antiviral antioxidant anti-microbial, anti-inflammation, anti-cancer, and anti-diabetic.
	8.7	Caffeic acid (3,4-Dihydroxycinnamic acid)	3.14	180.16	$C_9H_8O_4$	
	10.8	Ferulic acid (3-(4-hydroxy-3-methoxyphenyl) acrylic acid)	6.88	194.186	$C_{10}H_{10}O_4$	

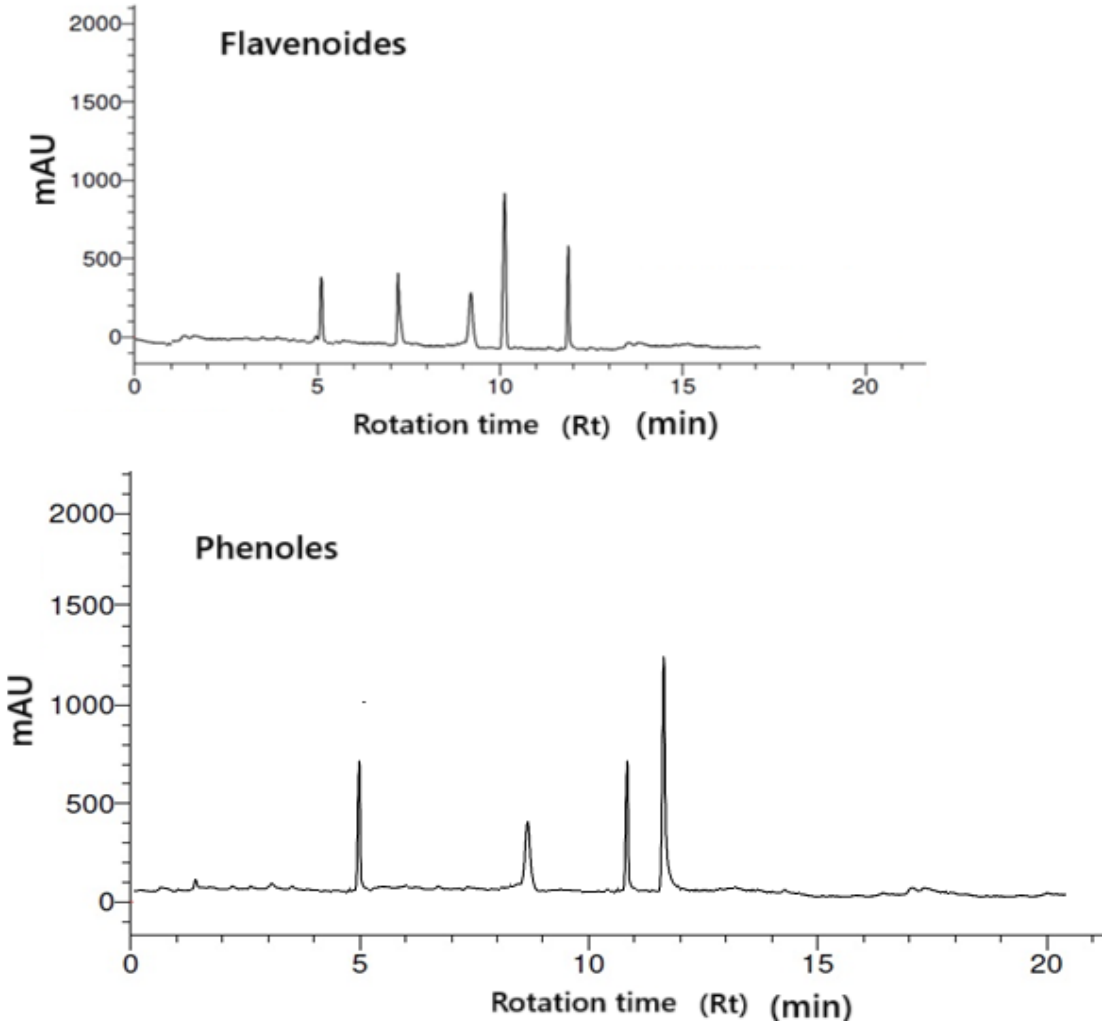

Fig.6: Photographic showing the peaks of abundance of active compounds of extract at different retention time.

Antiviral Activity of DPKME:

Minimum Inhibition Concentration (MIC):

The MIC **of** DPKME was 700.µg/mL and maximum Non-Toxic Dose (MNTD) was 200 (µg/ml) by MTT assay which did not appear any morphological change on Vero cells which % cell viability 100% (Table 7 and Fig. 7). As well as approximately 50 % of cells showed CPE infected with HSV -1 at 700 (µg/ml) SME was assayed using (MTT) assay.

Cytotoxicity: DPKME shows cytotoxicity effect on Vero cell (Vero E-6) at concentration of 700 µg/ml this concentration reduces cell availability to 50% after incubation and reaches cell confluency of 80 as shown in Figure (7).

Antiviral Activity of DPKME dissolved in DMSO showing antiviral activity against isolated HSV-1 with IC50 concentration of 100(µg/ ml) which inhibit HSV-1 after incubation in cell line to half amount of first stage, as Shown in Figure (7).

Table 7: MIC Antiviral Activity of DPKME dissolved in DMSO showing antiviral activity against isolated HSV-1 of DPKME Ug/ml using MMT assay by Percentage of Vero cell viability.

	DPKME conc. µg/mL										
	1000	900	800	700	600	500	400	300	200	100	50
OD	0.034	0.058	0.065	0.072	0.109	0.162	0.195	0.201	0.202	0.210	0.211
% cell viability	15.6	21.4	34.5	40.3	45.5	52.4	67.5	78.2	85.5	100	100
% HSV-1 inhibition	85.4	80.7	78.2	75.8	70.3	67.5	63.7	58.6	51.4	0.0	0.0

OD = Optical density of cell control = 0.35 % of DPKME effects on survival Vero cell line
OD = Optical density of cell control .0.32

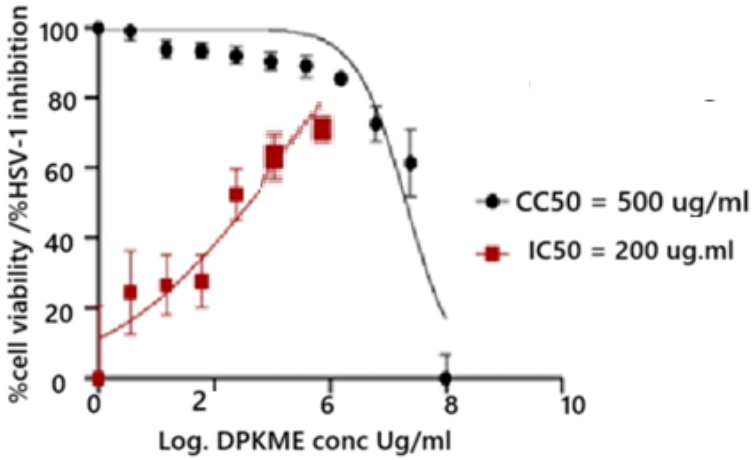

Fig. 7: . Photographic showing MIC and **antiviral activity** of DPKME against isolated HSV-1 using MMT assay by Percentage of Vero cell viability.

3- HSV Replication:

The DPKME was reduced in HSV replication in Vero cell with HSV 40.66% (Table,7 & Fig. 7). In addition, this effect of DPKME was similar to that of 3TC (125 lg/ml; positive control). However, DPKME did not have such inhibitory effect on the intracellular HSV-DNA level (Fig. 7). The mRNA transcript level of HSV Ag was reduced significantly by DPKME at 9.0 log CFU/ml, which had better effect than 3TC (Fig. 7). However, there were insignificant variances in the gene expression levels of HRV and HSV Ag (Fig. 8).

Table 8: Percentage of cytopathic effect using inhibition assay of DPKME against HSV.

	HSV-1 Titer (log 10)	**Titer post treatment**	**Log. Different**	**% reduction**
CG	6.0	5.8	0,2	24
DPKKME	6.0	4.5	1,5	24
CG@ DPKKME	6.0	4.2	1,5	24

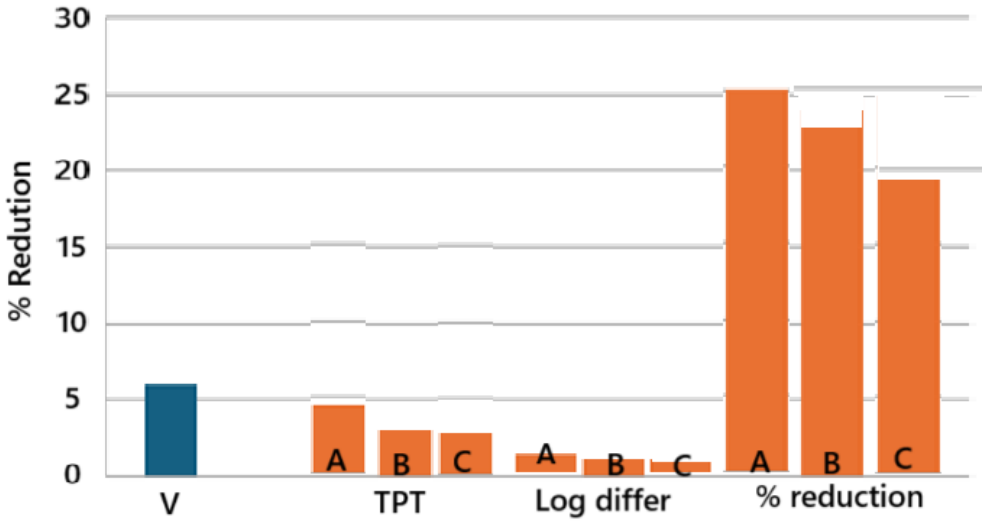

Fig 8. Histogram showing the effect of CG(A) , DPKKME (B), CG@ DPKKME(C).

Objective Assessments of The Quality of Facial Skin Gel:

The results of the objective biophysical assessment are presented in Table 3 including all four skin quality parameters, after treatment with CG@DPKME. All results showed significant improvement on both day 15 and day 30 compared to before treatment ($P < 0.05$). Showing improvements in skin quality (skin type, skin texture, Skin color tone and Skin moisture), (Table 9).

1 Facial Skin Type Results:

The average skin type improved on the 15th day by a rate of 55, and the improvement increased with use by 90% on the 30th day of use compared to what it was before use, with a change in the skin Dry percentage from 75 to 0% on the 30th day of use. The percentage of skin Oily changed from 10 to 0% on the 30th day of use. The percentage of skin combinations changed from 20 to 10% on the 30th day of use

2- Skin Color Tone Results:

The average skin Bright improved on the 15th day by a rate of 5 and the improvement increased by 95% on the 30th day of use compared to what it was before use, with a change in the skin Dark percentage from 25 to 0% on the 30th day of use. The percentage of Normal skin changed from 70 to 5% on the 30th day of use.

3 - Skin Texture Results:

An average improvement in skin texture on day 15 by a rate of 55%, and the improvement increased with use by 75% on day 30 of use compared to what it was before use, with a change in the skin Smooth percentage from 15 to 75% on day 30 of use. The percentage of normal skin changed from 75 to 25% on the 30th day of use. The percentage of skin rough changed from 10 to 0% on the 30th day of use

4- **Skin Moisture:**

An average improvement in skin moisture on day 15 by a rate of 50, and the improvement increased with use by 75% on day 30 of use compared to what it was before use, with a change in the percentage of skin moisture from 5 to 75% on day 30 of use. The skin normal percentage changed from 30 to 10% on the 30th day of use. The percentage of skin dryness changed from 65 to 15% on the 30th day of use.

Table 9. Subject demographics and percentage of improvement characteristics

Subject characteristics		Pre Treatment (n =20)	Post treatment					
			15 days			30 days		
			(n=%)	Change rate (%)	p-values	(n=%	Change rate (%)	p-values
Facial skin type (n=20)	Dry	13 (75.0)	4 (20.0)	55	< 0.001	0 (0.0)	90	< 0.001
	Normal	1 (5.0)	12 (60.0)		< 0.001	18(90.0)		< 0.001
	Oily	2 (10.0)	2 (10.0)		< 0.050	0 (0.0)		< 0.001
	Combination	4 (20.0)	2(10.0)		< 0.005	2 (10.0)		< 0.005
Skin color tone (n=20)	Bright	1 (5.0)	14 (70.0)	70	< 0.001	19(95.0)	95	< 0.001
	Normal	14 (70.0)	1 (5.0)		< 0.001	1 (5.0)		< 0.001
	Dark	5 (25.0)	5 (25.0)		< 0.045	0 (0.0)		< 0.001
Skin color tone(n=20)	Smooth	3 (15.0)	11 (70.0)	55	< 0.001	15(75.0)	75	< 0.001
	Normal	15 (75.0)	4 (20.0)		< 0.005	5 (25.0)		< 0.001
	Rough	2 (10.0)	2 (10.0)		< 0.054	0 (0.0)		< 0.001
Skin texture (n=20)	Moist	1 (5.0)	10 (50.0)	50	< 0.001	15 (75.0)	75	< 0.001
	Normal	6 (30.0)	2 (10.0)		< 0.005	2 (10.0)		< 0.001
	Dry	13 (65.0)	8 (40.0)		< 0.005	3(15.0)		< 0.005

*Note: Paired t-tests were conducted to obtain the p-value, unless marked with ^, which indicates the use of the Wilcoxon signed-rank test. Statistical significance was determined as $p < 0.05$, with a comparison made to the value before treatment.
- The percent change (improvement characteristics) was calculated using the formula: (values before treatment– values after treatment)/values after treatment ×100.

DISCUSSION

Herpes virus disease prevalence has been identified depending on distanced clinical symptoms like acute inflammation and tissue skin damage that required hospitalization it collected from woman and man at different hospitals belong Qalyubia and Cairo governorates. As well as it detected in concentrated clinical pus and fluid of using ILFST test (Abdel-Shaheed, *et al.,*2021).

Four HSV clinical specimens showing distanct acute severe infection were isolated that showed cytopathic effect assayed on Vero cells using MTT on the Vero cell which showed plaques and cytoplasmic inclusion bodies and different titration 1.2x106, 3.0x106, 4.2x106 and 7.5x105 plaque forming units per milliliter after 4 days post infection The HRVq1, HRVq2, HRVC1 and HRVC2 respectively (El-Kholy, *et al.,*2019 and El-Far, *et al.,*2021).

The integrity and quantity of the purified total DNA extracted from infected Vero cells with four HSV isolates confirmed by agarose gel electrophoresis and UV spectrophotometer. Amplification of glycoprotein D region DNA of the HSV genome of four HSV isolates were amplified by PCR and specific primer sets. The PCR product has been confirmed utilizing agarose gel electrophoresis with the expected size calculated ≈ 400 bp related to leader molecular DNA (Ahmad, *et al.,*2022 and Gomaa, *et al.,*2024).

Multiple sequence alignment (MSA) of glycoprotein D region of HSVQ1 isolate was displayed Max Score or total Score ranged 983 to 977 total score, varied to 97.55, Quarry Cover 100%, E. value 0.0 percentage identity ranged 97.73 to 97.55. The phylogenetic tree showed two clusters in which HSV of HSVQ1 isolate with 100 HSV isolates recorded on GenBank. The similarity percentage was 99.55% (Shakiba, *et al.,*2011). The genetic distance was 0.001 of HSVQ1 isolate Query_7241739 related to 100 HSV isolates recorded on Genback based on multiple alignment of nucleotide sequence GenBank (Shakiba, *et al.,*2011).

Active gradient compounds of DPKME: The phytochemical composition of DPKME were determined by GC-mass analysis (Abo-elmaaty, *et al.* 2016). Analysis firmed that contains 37 peaks at different retention times consists of 37 active compounds, consisting essential and

effective components that exhibit numerous bioactive activities (El- Far *et al.* 2021). These components include Flavonoids. (Rutin, Querestin, Luteolin, Kampfero, Catechin) and phenols (Syringic, Caffeic, Ferulic and Epicatechin) These components showed antiviral, anti-inflammatory, antioxidant biological activities compound the graph show condensed peak of compounds precipitated, results estimated by determining the Molecular weight and molecular formula through PubChem and PubMed website (Kumar, *et al.* 2019).

The MIC of DPKME was 700.µg/mL and maximum Non-Toxic Dose (MNTD) was 200 (µg/ml) by MTT assay which did not appear any morphological change on Vero cells which % cell viability 100%. As well as approximately 50 % of cells showed CPE infected with HSV -1 at 700 (µg/ml) SME was assayed using (MTT) assay. Cytotoxicity: DPKME show cytotoxicity effect on Vero cell (Vero E-6) at concentration of 700 µg/ml this concentration reduces cell availability to 50% after incubation and reach cell confluency of 80 (Aljaloud, *et al.,*2020)

Antiviral Activity of DPKME dissolved in DMSO showing antiviral activity against isolated HSV-1 with IC50 concentration of 100(µg/ ml) which inhibit HSV-1 after incubation in cell line to half amount of first stage.

The DPKME was reduction HSV replication in Vero cell with HSV 40.66%. However, DPKME did not have such inhibitory effect on the intracellular HSV-DNA level. The mRNA transcript level of HSV Ag was reduced significantly by DPKME at 9.0 log CFU/ml, which had better effect than 3TC. However, there were insignificant variances in the gene expression levels of HRV and HSV Ag. All results of the objective biophysical assessments including all four skin quality parameters, after use the CG@DPKME showed significant improvement on both day 15 and day 30 compared to before use Showing improvements in skin quality (skin type, skin texture, Skin color tone and Skin moisture). The quality of cosmetic gel has a profound effect on attractiveness, motivating many individuals to seek aesthetic procedures to improve their appearance, and cure them from infection. A panel of global beauty experts identified four traits, known as Emergent Perceptual Categories (EPCs), that describe good skin quality across ethnicities: (1) skin radiance, (2) skin firmness, (3) skin evenness, and (4) even skin tone [1]. The perception of good skin quality is shaped by several traits or EPCs, which are interconnected and can influence each other (Goldie, *et al.,* 2021 and Park, *et al.,* 2025).

CG@DPKME is used regularly for facial rejuvenation and has a short recovery time and no side effects when used correctly by skilled practitioners and can treat a range of skin infections (Belmontesi *et al.,*2018), a low-viscosity formulation formulated using DPKME with incorporated glycerin and) Kleine-Börger *et al.,* 2022). It is suitable for superficial coatings in facial rejuvenation . Glycerin is a humectant known for its ability to enhance skin hydration, improve skin mechanical properties, reduce trans epidermal water loss (TEWL), prevent lipid phase transition in the stratum corneum, provide anti-irritant and anti-inflammatory effects, accelerate wound healing, and exhibit antimicrobial actions (Fluhr, *et al.,* 2008). Improvements in skin elasticity, firmness, tone, radiance, hydration, fatigue, roughness and redness have been observed for up to 30 days after use. The study reported a favorable safety profile, with both subjects and investigators expressing high satisfaction with the results (Korponyai *et al.,* 2017).

Declarations:

Ethical Approval: The research proposal was reviewed and approved by the Scientific Research Ethics Committee of the Faculty of Science, Suez Canal University, with reference number Rec192/2023.

Authors Contributions: Nader A. Ismail, Hanaa H. Gomaa and Khalid A.

EEldougdoug.conceptualized the study. Dalia Y. Zaghloul ; Alaa eldin R. Ahmed; and Khalid A. Eldougdoug carried out the methodology, data collection, and analysis. A Dalia Y. Zaghloul ; and Khalid A. Eldougdoug prepared the initial manuscript draft. Nader A. Ismail Hanaa H. Gomaa and Khalid A. Eldougdoug reviewed and edited the manuscript. All authors approved the final published version

Consent for publication: All authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the Virology Labs, Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Cairo Egypt and botany and microbiology Department, faculty of science, suez canal university, Ismailia, Egypt where the work has been carried out before the work is submitted.

Competing Interest: The authors declare no conflict of interest.

Data availability Statement: All data are presented within the article.

Funding: This research received no external grants or funding.

Acknowledgment: We gratefully acknowledge the Virology Lab, Agricultural Microbiology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, for their significant support and for furnishing the necessary resources for this scientific research.

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