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Original Article

# GC/MS Profiling of *Becium grandiflorum* Essential Oil and Evaluation of Its Antiviral Activity

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## ABSTRACT

Genus *Becium* of the Lamiaceae family encounters numerous medicinally and economically valued aromatic plants. The hydrodistilled essential oil of *Becium grandiflorum* (Lam.) Pic. Serm. aerial part was analyzed using GC/MS. A total of 32 compounds amounting to 99.68% of the essential oil were identified. Oxygenated diterpenes constituted the largest share (43.45%) in the studied oil followed by sesquiterpenes hydrocarbons (34.24%). Pimara-7,15-dien-3-ol (20.58%), 3-*α*-hydroxymanool (14.07%), caryophyllene (8.17%), sandaracopimaradiene (7.98%), Selina-3,7 (11)-diene (5.61%), and germacrene D (4.95%) are the chief identified compounds in *B. grandiflorum* essential oil. The essential oil was assessed for its antiviral potential against herpes simplex virus 1 (HSV-1) at a non-cytotoxic concentration using a cytopathic effect (CPE) inhibition assay on the Vero cell line. *B. grandiflorum* essential oil exhibited a pronounced *in vitro* cytotoxicity against Vero cells with 50 % cytotoxic concentration ($CC_{50}$) of 7.75 ± 0.84 µg/mL and weak anti-HSV-1 potency with percent inhibition of 21.36 ± 2.13 at a maximum non-toxic concentration of 1 µg/mL.

**Keywords:** *Becium grandiflorum; essential oil; GC/MS; antiviral; MTT assay.*

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## 1. Introduction

Lamiaceae (Labiatae) traditionally named as the mint family, is a family of flowering plants that encompasses more than 3000 species. *Salvia*, *Scutellaria*, and *Stachys* are among the largest genera of Lamiaceae. Moreover, thyme, oregano, mint, basil, and rosemary are the most prevalent culinary spices of this family. These plants are native to the Mediterranean region. They are herbs or shrubs with a characteristic aroma on account of the presence of a considerable percentage of essential oil **[1, 2]**. Essential oil is a complex combination of liquid, volatile chemical constituents that are mainly obtained from plant material through distillation using water and/or steam or mechanical processes **[3]**. Moreover, essential oils are composed of phenylpropanoids, low molecular weight hydrocarbon derivatives, or terpenoids. The later encounters the major class of chemical constituents of essential oils. In addition to their antimicrobial, antifungal, antiviral, cytotoxic, and insecticidal potency, they are broadly used in the cosmetics industry, perfumery, and aromatherapy **[4]**. Worldwide, there has been a notable surge in the use of plant extracts or essential oils for the treatment and

prevention of numerous ailments due to their inestimable safety and efficacy profiles **[5]**. Literature review revealed that various plants of the genus *Becium* which is a synonym of *Ocimum* **[6, 7]** are a rich source of essential oils with valuable biological activities **[8]**. *Ocimum basilicum* essential oil along with some identified monoterpenes such as camphor, thymol, and 1, 8-cineole exhibit antiviral potential against bovine viral diarrhea virus (BVDV) **[9]**. Moreover, eugenol, one of the chief constituents of different *Ocimum* species essential oils, possesses antiviral power against herpes simplex viruses type 1 and 2 (HSV-1 & 2) and human immunodeficiency virus (HIV) by inhibiting viral replication **[8]**.

HSV, or Herpes Simplex Viruses, are viruses with double-stranded DNA that are categorized into two antigenic types: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). HSV-1 is one of the primary causes of human infections affecting more than 60% of the global population. It can induce orofacial infections (herpetic labialis), herpetic keratitis, and life-threatening encephalitis **[10]**. It is mainly transmitted through oral-to-oral contact and to less extent through oral-genital contact [11]. The virus can establish a latency period and can be reactivated in certain conditions leading to significant complications in immunocompromised patients **[12]**.

*Becium grandiflorum* (Lam.) Pic. Serm. is an aromatic perennial woody shrub belonging to the family Lamiaceae that grows up to one meter tall. It is cultivated on the rocky slopes of the altitudes of Ethiopia and is commonly known as Tebeb. In traditional medicine, it was commonly used against several ailments such as malaria, spider bites, swellings, influenza, and respiratory depression. Moreover, citizens of the Tigray region usually apply crushed leaves on the wound area to aid in wound healing which was recently evidenced by a research article **[13]**. Literature review revealed that *B. grandiflorum* ethanolic extract exhibits blood sugar-lowering potential **[7]** in addition to its antimicrobial activity against S*taphylococcus aureus* and *S. pneumonia* [**14]**.

This study aimed to explore the chemical profile of the essential oil of *B. grandiflorum* cultivated for the first time in Egypt using gas chromatography coupled to mass spectrometry (GC/MS) along with evaluation of its *in vitr*o antiviral activity against HSV-1 using cytopathic effect (CPE) inhibition assay on African green monkey kidney (Vero) cell line.

## 2. Materials and Methods

### 2.1. Plant Collection and Authentication

The aerial part of *B. grandiflorum* (Lam.) Pic.Serm. **[15]** was collected at the flowering stage in July 2021 from Eng. Khalid El Haddad's farm, Giza, Egypt. The plant material's identity was verified by Eng. Therese Labib, Botanical Consultant and Specialist at Orman and Qubba Botanical Gardens. An authentic specimen (PHG-P-BG-325) was preserved in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

### 2.2. Hydrodistillation of the Essential Oil and GC/MS Analysis

The aerial part of *B. grandiflorum* (250 g) was hydrodistilled for 4 h in Clevenger-type apparatus. In accordance with the Egyptian pharmacopeia, anhydrous sodium sulfate was utilized to dehydrate the obtained essential oil that was reserved at 4 °C for subsequent analysis **[16]**. Based on the fresh plant weight, the yield was calculated (yield % v/w). For separation and identification of the chemical constituents, analytical GC/MS was used (Shimadzu GCMS-QP2010, Koyoto, Japan) supplied with fused bonded Rtx-5MS column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Restek, USA) and supported with a split/splitless injector. The column's initial temperature was maintained

(isothermal) for 3 min at 50 °C followed by linear temperature increase at a rate of 5 °C/min to reach 300 °C then the temperature was maintained (isothermal) at 300 °C for 10 min. The temperature of the injector was held constant at 280 °C. Helium was employed as a carrier gas, flowing at a rate of 1.37 mL/min. All the mass spectra were captured under the following settings: The filament emission current was 60 mA; the ionization voltage was 70 eV; and the ion source temperature was 220 °C. With split mode, diluted samples (1% v/v) were injected (split ratio 1:15). Essential oil chemical constituents were identified by comparing the Kovats index (KI) on the Rtx-5 column to the available literature **[17]**, research articles, and computer library (NIST-11 mass spectral library) **[18-20]**.

### 2.3. Cell Culture and Virus Propagation

Vero cells were collected from the American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM) was supplied for the cultivation of Vero cells which was provided with 1% L-glutamine, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer, 50 µg/mL Gentamycin and 10% heat-inactivated fetal bovine serum (FBS). The cell cultures were kept in a humidified environment at 37 °C containing 5% $CO_2$ and were subcultured twice a week **[21]**. The cytopathogenic HSV-1 virus was cultured and assessed in confluent Vero cells **[22]**. By utilizing The Spearman-Karber technique, the number of infectious viruses was counted by calculating the 50% tissue culture infectious dose ($TCID_{50}$) with 20 µl of inoculum in each well and eight wells for each dilution **[23]**.

### 2.4. Cytotoxicity Assay

Cytotoxicity assay was conducted at the Regional Center for Mycology and Biotechnology (RCMB, Al-Azhar University, Cairo, Egypt). Cellular vitality and proliferation were assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay **[24]**. Vero cells were grown in 96-well plates containing 100 µL of the growth medium at a concentration of ($2 \times 10^5$ cells/mL) and incubated for 24 h to allow cellular adherence. After incubation, Vero cells were treated with different concentrations of the tested essential oil dissolved in a fresh medium. Using a multichannel pipette, two-fold dilutions in the row of the tested sample at a concentration range (3000 µg/mL to 2 µg/mL) were supplied to the flat-bottomed multiwell plates (Falcon, Jersey, NJ, USA). For 48 h, the 96-well plates were kept at 37 °C in a humidified atmosphere supplied with 5% $CO_2$ **[24]**. After completion of the incubation period, the culture medium was eliminated and the multiwell plates were refilled with 100 µL of fresh culture medium. 10 µL of 5 mg/mL MTT dissolved in phosphate buffer saline (PBS) was introduced to each well including untreated control and incubated for 4 h at 37 °C. Following the incubation period, the medium was eliminated and 50 µL of dimethyl sulfoxide (DMSO) was introduced to each well and properly stirred using a pipette before incubation under the same conditions for 10 min. The absorbance was recorded at 590 nm using a microplate reader (SunRise, TECAN, Inc, USA) **[24]**. The percentage of cell viability was computed as [(ODt/ODc)] x 100% where ODt represents the mean optical density of the test sample while ODc is the mean optical density of control cells. A relationship was plotted between the number of viable cells and the concentrations of the tested essential oil to draw the survival curve of Vero cells. The 50% cytotoxic concentration ($CC_{50}$) was estimated using GraphPad Prism software (San Diego, CA. USA) by plotting the dose-response curve for each concentration. The sample's maximum non-toxic concentration (MNTC) was measured to be

utilized in the assessment of antiviral potency.

### 2.5. Screening for Antiviral Activity

Cytopathic effect (CPE) inhibition assay was used to assess the antiviral activity of the tested essential oil at the Regional Center for Mycology and Biotechnology (RCMB, Al-Azhar University, Cairo, Egypt). This method was employed to demonstrate certain suppression of cellular activity. CPE in susceptible Vero cells was measured by the MTT dye uptake method **[25, 26]**. In a multiwell microtiter plate, Vero cell monolayers at a cellular concentration ($2x10^5$ cells/mL) were seeded. This was followed by 24 h incubation period at 37 °C in a humidified chamber supplied with 5% $CO_2$. The medium was eliminated and replaced with fresh DMEM before being inoculated with $10^4$ doses of HSV-1. Various concentrations of the tested essential oil maintained in a fresh medium were supplied to the wells and kept at 37 °C for 48 h. As a control, non-infected and infected untreated Vero cells were utilized. The suppression of the CPE and the cellular protection provided by the tested essential oil relative to the control were used to measure antiviral potency. Three independent tests with four replicates per treatment were evaluated. In this experiment, Acyclovir was used as a positive control. Cells' vitality was evaluated using a colorimetric MTT assay after the completion of the incubation period. **[24]**. The rate of viral inhibition was estimated as: $[(A - B)/ (C - B)] \times 100\%$ where A is the absorbance of treated virus-infected cells, while B is the absorbance of infected untreated control and C is the absorbance of the cell control.

## 3. Results and Discussion

### 3.1. Chemical Composition of *B. grandiflorum* Essential Oil

The aerial part of *B. grandiflorum* yielded 0.08% v/w essential oil (EO). The essential oil was lighter than water with pale yellow color. The GC/MS chromatogram is provided in **Fig. 1.** The identified components of *B. grandiflorum* essential oil were listed in **Table 1** in accordance with their order of elution. Thirty-two chemical constituents were identified representing 99.68% of the total chromatographic area. Oxygenated diterpenes and sesquiterpene hydrocarbons constitute the predominant classes of compounds in *B. grandiflorum* essential oil representing 43.45% and 34.24%, respectively. The predominance of oxygenated diterpenes in the tested sample is dissimilar to other EOs isolated from *O. basilicum* and *O. gratissimum* harvested from various countries in which oxygenated monoterpenes are the major class of identified compounds **[19, 20, 27, 28]**. However, the presence of sesquiterpene hydrocarbons in the second rank is in line with other reports on *O. basilicum* cultivated in Iran and *O. gratissimum* obtained from Colombia **[29, 30]**. Moreover, it was reported that two sesquiterpene hydrocarbons; *β*-caryophyllene and Germacrene D were dominant in 4 species of *Ocimum* (*O. gratissimum, O. basilicum* var. *thyrsiflorum, O. citriodorum* and *O. sanctum* var. *Shyama*) cultivated in Thailand **[31]**. Diterpene hydrocarbons come in the third subclass with 12.31% followed by fatty acid-derived volatiles (4.97%) and oxygenated sesquiterpenes (4.43%). The major identified constituents were Pimara-7,15-dien-3-ol (20.58%), 3-*α*-Hydroxy-manool (14.07%), Caryophyllene (8.17%), Sandaracopimaradiene (7.98%), Selina-3,7(11)-diene (5.61%), and Germacrene D (4.95%). **Fig. 2.** represents the major identified chemical components. Analysis of *B. grandiflorum* EO cultivated in Ethiopia demonstrated that the major constituents are (Z)-*p*-ocimene (17.6%), myrcene (8.4%), limonene (8.0%), and *β*-caryophyllene (7.6%) **[18]**. The qualitative and quantitative variations in the EO content of *B. grandiflorum* cultivated in Ethiopia *vs*. that newly

cultivated in Egypt may be attributed to variations in the collection time and cultivation conditions e.g., the soil type, pH and content of organic matter which can affect the chemical constituents of the plant **[32]**. It is supported by the fact that Ethiopian *B. grandiflorum* natively grows on the sandy soil of the altitudes and slopes **[13]** in contrast to the newly cultivated in Egypt that grows in muddy soil.

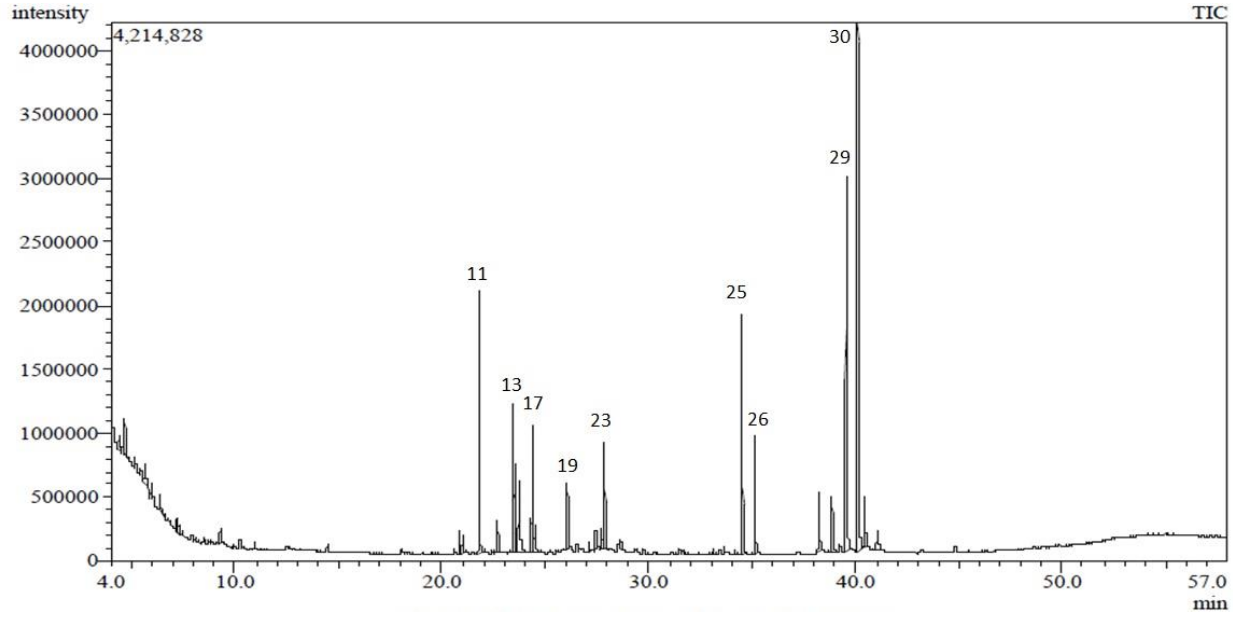


**Fig. 1.** GC/MS chromatogram of *B. grandiflorum* EO

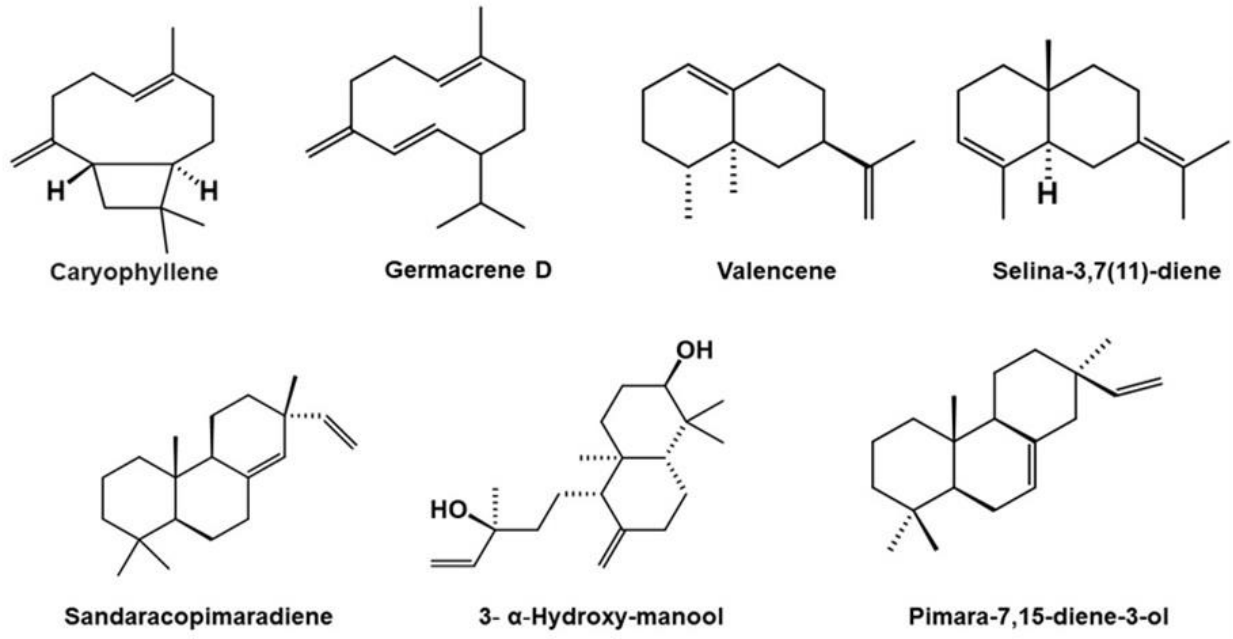


**Fig. 2.** Major identified compounds in *B. grandiflorum* EO

**Table 1. Volatiles identified from the essential oil of *B. grandiflorum***

| Peak # | Component | Molecular formula | Molecular weight | RT (Min) | Relative percentile | KI | |
|---|---|---|---|---|---|---|---|
| | | | | | | Obsd. | Lit. |
| 1 | Acetic acid, butyl ester | $C_6H_{12}O_2$ | 116 | 4.35 | 0.78 | 807 | 807 |
| 2 | Ethyl cyclohexane | $C_8H_{16}$ | 112 | 4.69 | 1.03 | 819 | 826 |
| 3 | *p*-Dimethyl benzene | $C_8H_{10}$ | 106 | 5.61 | 1.06 | 853 | 860 |
| 4 | Nonane | $C_9H_{20}$ | 128 | 6.36 | 0.66 | 880 | 900 |
| 5 | *trans*-1-Methyl-4-Ethylcyclohexane | $C_9H_{18}$ | 126 | 6.54 | 0.32 | 887 | 899.8 |
| 6 | propyl cyclohexane | $C_9H_{18}$ | 126 | 7.19 | 0.55 | 910 | 912.2 |
| 7 | Linalool | $C_{10}H_{18}O$ | 154 | 7.32 | 0.28 | 915 | 1062 |
| 8 | Decane | $C_{10}H_{22}$ | 142 | 9.29 | 0.57 | 987 | 1000 |
| 9 | (-)-*β*-Bourbonene | $C_{15}H_{24}$ | 204 | 20.85 | 0.88 | 1374 | 1385 |
| 10 | *β*-elemene | $C_{15}H_{24}$ | 204 | 21.03 | 0.64 | 1380 | 1391 |
| 11 | Caryophyllene | $C_{15}H_{24}$ | 204 | 21.80 | **8.17** | 1408 | 1408 |
| 12 | Humulene | $C_{15}H_{24}$ | 204 | 22.71 | 1.08 | 1444 | 1455 |
| 13 | Germacrene D | $C_{15}H_{24}$ | 204 | 23.44 | **4.95** | 1472 | 1472 |
| 14 | 4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7 octahydronaphthalene | $C_{15}H_{24}$ | 204 | 23.49 | 2.57 | 1474 | 1485 |
| 15 | *β*-Selinene | $C_{15}H_{24}$ | 204 | 23.59 | 0.76 | 1478 | 1476 |
| 16 | Valencene | $C_{15}H_{24}$ | 204 | 23.76 | **4.05** | 1485 | 1485 |
| 17 | Selina-3,7(11)-diene | $C_{15}H_{24}$ | 204 | 24.39 | **5.61** | 1510 | 1518 |
| 18 | *δ*-Cadinene | $C_{15}H_{24}$ | 204 | 24.49 | 1.21 | 1514 | 1523 |
| 19 | Viridiflorol | $C_{15}H_{26}O$ | 222 | 26.06 | 2.28 | 1574 | 1590 |
| 20 | Cadine-1,4-diene | $C_{15}H_{24}$ | 204 | 27.13 | 0.36 | 1618 | 1540 |
| 21 | *Tau*-Cadinol acetate | $C_{17}H_{28}O_2$ | 264 | 27.43 | 1.25 | 1631 | 1770 |
| 22 | *Tau*-Cadinol acetate | $C_{17}H_{28}O_2$ | 264 | 27.77 | 0.9 | 1646 | 1770 |
| 23 | Eudesma-4(14),11-diene | $C_{15}H_{24}$ | 204 | 27.89 | **3.96** | 1652 | 1531 |
| 24 | Unknown | - | - | 28.64 | 0.33 | 1684 | - |
| 25 | Sandaracopimaradiene | $C_{20}H_{32}$ | 272 | 34.55 | **7.98** | 1955 | 1951 |
| 26 | Isopimaradiene | $C_{20}H_{32}$ | 272 | 35.15 | **4.33** | 1984 | 1994 |
| 27 | Sclareol | $C_{20}H_{36}O_2$ | 308 | 38.22 | 2.59 | 2150 | 2194 |
| 28 | Sandaracopimaral | $C_{20}H_{30}O$ | 286 | 38.90 | 2.29 | 2187 | 2185 |
| 29 | 3-*α*-Hydroxy-manool | $C_{20}H_{34}O_2$ | 306 | 39.54 | **14.07** | 2222 | 2273 |
| 30 | Pimara-7,15-dien-3-ol | $C_{20}H_{32}O$ | 288 | 40.14 | **20.58** | 2255 | 2253 |
| 31 | Kaurenol | $C_{20}H_{32}O$ | 288 | 40.45 | 2.59 | 2272 | 2302 |
| 32 | Isopimarol | $C_{20}H_{32}O$ | 288 | 41.04 | 1.33 | 2304 | 2305 |
| | Total identified % | | | 99.68 | | | |
| | Oxygenated diterpenes % | | | 43.45 | | | |
| | Sesquiterpene hydrocarbons % | | | 34.24 | | | |
| | Diterpene hydrocarbons % | | | 12.31 | | | |
| | Fatty acid-derived volatiles % | | | 4.97 | | | |
| | Oxygenated sesquiterpenes % | | | 4.43 | | | |
| | Oxygenated monoterpenes % | | | 0.28 | | | |

$KI_{obsd.}$: Kovats indices evaluated experimentally on Rtx-5 column relative to C8–C28 *n*-alkanes series.

$KI_{lit}$: Reported Kovats indices.

### 3.2. Cytotoxicity Evaluation

Assessment of cytotoxicity is a crucial step before the evaluation of a potential antiviral agent by CPE inhibition assay. To establish the maximum non-cytotoxic concentration of *B. grandiflorum* EO to be employed for possible antiviral use, an MTT assay was conducted against Vero cells. MTT assay is one of the most widely used colorimetric assays for the assessment of cell viability that depends on the reduction of water-soluble tetrazolium dye (MTT) by mitochondrial dehydrogenase enzyme in metabolically active cells **[33]**. Vero cell proliferation was dose-dependently suppressed

by EO of *B. grandiflorum* as illustrated in **Fig. 3**. An *in vitro* cytotoxicity study reported that herbal extract having $IC_{50}$ value < 20 µg/mL is considered cytotoxic **[34]**. In the present study, the oil exhibited significant cytotoxic activity against Vero cells with $CC_{50}$ as low as 7.75±0.84 µg/mL. The high cytotoxic activity of the *B. grandiflorum* EO could be related to the dominance of oxygenated diterpenes that provide powerful cytotoxicity **[35, 36]**.

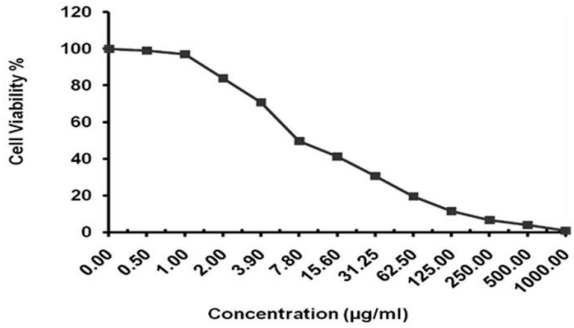


**Fig. 3.** Viability of Vero cells treated with different concentrations of *B. grandiflorum* EO

### 3.3. Screening for Antiviral Activity

The potential antiviral action of *B. grandiflorum* EO was performed by viral CPE inhibitory method on Vero cells and observed using MTT assay. **Table 2** shows the anti-HSV-1 activity of *B. grandiflorum* EO compared to acyclovir as a reference antiviral agent. At a maximum non-toxic concentration of the EO (1 µg/mL), the mean CPE inhibition % was 21.36±2.13, while that of acyclovir was 96.03±1.14. This result may indicate a weak anti-HSV-1 potential of *B. grandiflorum* EO. These findings are in agreement with another report **[37]** that evaluated the antiviral activity of *O. campechianum* EO against the Human herpes virus (HSV-1& HSV-2) and found that the oil has no activity against HSV-1 but showed inhibitory activity against HSV-2 with $EC_{50}$ 74.33±10.9 µg/mL. According to the literature, none of the major identified compounds except *β*-Caryophyllene has any reported anti-HSV-1 activity. Interestingly, one study supported the potential of *β*-Caryophyllene against HSV-1 with $IC_{50}$ of 0.25 µg/mL **[38]**. While *B. grandiflorum* EO did not show remarkable anti-HSV-1 potential, other species of the same genus have shown antiviral activities. Previous reports demonstrated that *O. gratissimum* leaves aqueous extract showed anti-HIV-1 with $IC_{50}$ of 1.1 mg/mL **[39]**. On the other hand, it was reported that the methanol and dichloromethane extracts of *O. americanum* L. exhibited antiviral potency against HSV-1 with $IC_{50}$ of 67.51 and 25.29 µg/mL, respectively [40]. Although various members of the genus *Ocimum* display antiviral properties, surprisingly *B. grandiflorum* showed weak anti-HSV-1 activity which may be attributed to the qualitative and quantitative diversity in the chemical constituents of *B. grandiflorum* EO.

**Table 2. Antiviral activity of *B. grandiflorum* essential oil against HSV-1**

| Sample | Tested concentration (µg/mL) | CPE inhibition (%) (Mean ± S.D.) |
|---|---|---|
| ***B. grandiflorum* EO** | 1 | 21.36±2.13 |
| **Acyclovir** | 2 | 96.03±1.14 |

## Conclusion

The current investigation is the first to report the volatile composition of *B. grandiflorum* newly cultivated in Egypt *via* GC/MS. Oxygenated diterpenes and sesquiterpene hydrocarbons are the chief classes of chemical components in *B. grandiflorum* EO. In addition to EO characterization, the anti-HSV-1 activity was evaluated. *B. grandiflorum* EO showed minor anti-HSV-1 power that may be attributed to variations in the chemical composition of the EO as a result of discrepancies in plant

harvesting age, genetic makeup, sunlight, place of origin, variety of soil, seasonal shifts, and method of oil extraction. Further analysis of *B. grandiflorum* EO across all seasons may yield different findings in terms of the chemical components and consequently biological activity.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent to publish**

Not applicable.

**Availability of data and materials**

The data generated or analyzed during this study are included in the main manuscript file.

**Competing interests**

The authors have no competing interests.

**Funding Statement**

**No funding source was received.**

**Authors' contributions**

Rola M. Labib supervised the work. Rola M. Labib, Noha Swilam, and Nehal Ibrahim contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Christeen Fahim. The first draft of the manuscript was written by Christeen Fahim and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**4. References**

1. Michel, J., N.Z. Abd Rani, and K. Husain. A review on the potential use of medicinal plants from Asteraceae and Lamiaceae plant family in cardiovascular diseases. Frontiers in pharmacology, 2020. 11: p. 852. doi: 10.3389/fphar.2020.00852
2. Karthıka, M. and A.R. Rasmi. Pharmacological potential of fungal endophytes associated with the genus *Ocimum* L. International Journal of Secondary Metabolite, 2023. 10(1): p. 1-10. doi: 10.3390/jof7020147
3. Kant, R. and A. Kumar. Review on essential oil extraction from aromatic and medicinal plants: Techniques, performance, and economic analysis. Sustainable Chemistry and Pharmacy, 2022. 30: p. 100829. doi: 10.1016/j.scp.2022.100829
4. Sharma, A., K. Gumber, A. Gohain, T. Bhatia, H.S. Sohal, V. Mutreja, et al., Importance of essential oils and current trends in the use of essential oils (aroma therapy, agro-food, and medicinal usage), in Essential Oils. 2023, Elsevier. p. 53-83. doi: 10.1016/B978-0-323-91740-7.00002-5
5. Nieto, G. Biological activities of three essential oils of the Lamiaceae family. Medicines, 2017. 4(3): p. 63. doi: 10.3390/medicines4030063
6. Hiltunen, R. and Y. Holm, Basil: the genus *Ocimum*. 1999: CRC Press. doi: 10.1201/9780203303771
7. Gebremeskel, L., K.B. Tuem, and T. Teklu. Evaluation of the antidiabetic effect of ethanolic leaves extract of *Becium grandiflorum* Lam. (Lamiaceae) in streptozotocin-induced diabetic mice. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 2020. 13: p. 1481. doi: 10.2147/DMSO.S246996
8. Tshilanda, D.D., E.M. Ngoyi, C.N. Kabengele, A. Matondo, G.N. Bongo, C.L. Inkoto, et al. Ocimum species as potential bioresources against COVID-19: a review of their phytochemistry and antiviral activity. International Journal of Pathogen Research, 2020. 5(4): p. 42-54. doi: 10.9734/IJPR/2020/v5i43014
9. Kubiça, T.F., S.H. Alves, R. Weiblen, and L.T. Lovato. *In vitro* inhibition of the bovine viral diarrhea virus by the essential oil of *Ocimum basilicum* (basil) and monoterpenes. Brazilian Journal of Microbiology, 2014. 45(1): p. 209-214. doi: 10.1590/S1517-83822014005000030
10. Harrell, T.L., D.J. Davido, and A.S. Bertke. Herpes Simplex Virus 1 (HSV-1) Infected Cell Protein 0 (ICP0) Targets of Ubiquitination during

Productive Infection of Primary Adult Sensory Neurons. International Journal of Molecular Sciences, 2023. 24(3): p. 2931. doi: 10.3390/ijms24032931

11. Garber, A., L. Barnard, and C. Pickrell. Review of whole plant extracts with activity against herpes simplex viruses in vitro and in vivo. Journal of Evidence-Based Integrative Medicine, 2021. 26: p. 2515690X20978394. doi: 10.1177/2515690X20978394
12. Chang, M.J., M.T. Liu, M.R. Chen, N. Li, Y.H. Zhao, S.X. Zhang, et al. Mendelian Randomization Analysis Suggests No Associations of Herpes Simplex Virus Infections With Systemic Lupus Erythematosus. Journal of Medical Virology, 2023. doi: 10.1002/JVM.28649
13. Beshir, K., W. Shibeshi, A. Ejigu, and E. Engidawork. *In-vivo* wound healing activity of 70% ethanol leaf extract of *Becium grandiflorum* Lam. (Lamiaceae) in mice. Ethiopian Pharmarmaceutical Journal, 2016. 32: p. 117-130. doi: 10.4314/epj.v32i2.3
14. Equar, G., G. Gebremedhin, A. Gebrekidan, T. Asmelash, and G. Yirga. Determination of antimicrobial activity and phytochemical screening of selected medicinal plants in the Tigray region of Northern Ethiopia. Momona Ethiopian Journal of Science, 2018. 10(2): p. 255-270. doi: 10.4314/mejs.v10i2.6
15. WFO. *Becium grandiflorum (Lam.) Pic.Serm.* 2023 [cited 2023 19 Feb ]; Available from: http://www.worldfloraonline.org/taxon/wfo-0000322633.
16. Pharmacopoeia, E. Egyptian Pharmacopoeia, General Organization for Governmental. Printing Office, Ministry of Health, Cairo, Egypt, 1984: p. 31-33
17. Adams, R.P., Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. 2017: Texensis Publishing.
18. Asfaw, N., H.J. Storesund, F. Tønnesen, A.J. Aasen, and L. Skattebøl. The volatile oil of *Becium grandiflorum* subsp. *grandiflorum* (Lam.) Pic. Serm. from Ethiopia. Journal of Essential Oil Research, 2000. 12(3): p. 325-326. doi: 10.1080/10412905.2000.9699527
19. Matasyoh, L.G., J.C. Matasyoh, F.N. Wachira, M.G. Kinyua, A.W.T. Muigai, and T.K. Mukiama. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. African Journal of Biotechnology, 2007. 6(6).
20. Rashid, A., F. Anwar, R. Qadir, R. Sattar, M.T. Akhtar, and B. Nisar. Characterization and Biological Activities of Essential Oil from Flowers of Sweet Basil (*Ocimum basilicum* L.) Selected from Different Regions of Pakistan. Journal of Essential Oil Bearing Plants, 2023: p. 1-13. doi: 10.1080/0972060X.2022.2155073
21. Vijayan, P., C. Raghu, G. Ashok, S. Dhanaraj, and B. Suresh. Antiviral activity of medicinal plants of Nilgiris. Indian Journal of medical research, 2004. 120: p. 24-29
22. Randazzo, W., J. Piqueras, J. Rodríguez-Díaz, R. Aznar, and G. Sánchez. Improving the efficiency of viability-qPCR for selective detection of infectious HAV in food and water samples. Journal of applied microbiology, 2018. 124(4): p. 958-964. doi: 10.1111/jam.13519
23. Pintó, R.M., J.M. Diez, and A. Bosch. Use of the colonic carcinoma cell line CaCo-2 for *in vivo* amplification and detection of enteric viruses. Journal of medical virology, 1994. 44(3): p. 310-315. doi: 10.1002/JVM.1890440317
24. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 1983. 65(1-2): p. 55-63. doi: 10.1016/0022-1759(83)90303-4
25. Hu, J. and G. Hsiung. Evaluation of new antiviral agents: I. *In vitro* perspectives. Antiviral research, 1989. 11(5-6): p. 217-232. doi: 10.1016/0166-3542(89)90032-6
26. Al-Salahi, R., I. Alswaidan, H.A. Ghabbour, E. Ezzeldin, M. Elaasser, and M. Marzouk. Docking

and antiherpetic activity of 2-amino benzo [de]-isoquinoline-1, 3-diones. Molecules, 2015. 20(3): p. 5099-5111. doi: 10.3390/molecules20035099

27. Dambolena, J.S., M.P. Zunino, A.G. López, H.R. Rubinstein, J.A. Zygadlo, J.W. Mwangi, et al. Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by Fusarium verticillioides. Innovative Food Science & Emerging Technologies, 2010. 11(2): p. 410-414. doi: 10.1016/j.ifset.2009.08.005
28. Pandey, A.K., P. Singh, and N.N. Tripathi. Chemistry and bioactivities of essential oils of some *Ocimum* species: an overview. Asian Pacific Journal of Tropical Biomedicine, 2014. 4(9): p. 682-694. doi: 10.12980/APJTB.4.2014C77
29. Hassanpouraghdam, M.B., A. Hassani, and M.S. Shalamzari. Menthone-and estragole-rich essential oil of cultivated *Ocimum basilicum* L. from Northwest Iran. Chemija, 2010. 21(1): p. 59-62
30. Benitez, N.P., E.M. Meléndez León, and E.E. Stashenko. Eugenol and methyl eugenol chemotypes of essential oil of species *Ocimum gratissimum* L. and *Ocimum campechianum* Mill. from Colombia. Journal of chromatographic science, 2009. 47(9): p. 800-803. doi: 10.1093/chromsci/47.9.800
31. Tangpao, T., H.-H. Chung, and S.R. Sommano. Aromatic profiles of essential oils from five commonly used Thai basils. Foods, 2018. 7(11): p. 175. doi: 10.3390/foods7110175
32. Adamczyk-Szabela, D. and W.M. Wolf. The impact of soil pH on heavy metals uptake and photosynthesis efficiency in *Melissa officinalis*, *Taraxacum officinalis*, *Ocimum basilicum*. Molecules, 2022. 27(15): p. 4671. doi: 10.3390/molecules27154671
33. Constante, C.K., J. Rodríguez, S. Sonnenholzner, and C. Domínguez-Borbor. Adaptation of the methyl thiazole tetrazolium (MTT) reduction assay to measure cell viability in Vibrio spp. Aquaculture, 2022. 560: p. 738568. doi: 10.1016/j.aquaculture.2022.738568
34. Sajjadi, S.E., M. Ghanadian, M. Haghighi, and L. Mouhebat. Cytotoxic effect of *Cousinia verbascifolia* Bunge against OVCAR-3 and HT-29 cancer cells. Journal of HerbMed pharmacology, 2015. 4(1): p. 15-19
35. Wei, W.J., B. Zhu, Y. Si, T. Guo, J. Kang, and L. Dai. Cytotoxic ent-Kaurane Diterpenoids from *Rabdosia Rubescens*. Chemistry & Biodiversity, 2022. 19(10): p. e202200497. doi: 10.1002/cbdv.202200497
36. Kongwaen, P., J. Boonsombat, S. Thongnest, S. Ruchisansakun, C. Mahidol, and S. Ruchirawat. Cytotoxic Isopimarane Diterpenoids from *Kaempferia koratensis* Rhizomes. Revista Brasileira de Farmacognosia, 2023: p. 1-7. doi: 10.1007/s43450-023-00359-w
37. Brand, Y.M., V.C. Roa-Linares, L.A. Betancur-Galvis, D.C. Durán-García, and E. Stashenko. Antiviral activity of Colombian Labiatae and Verbenaceae family essential oils and monoterpenes on Human Herpes viruses. Journal of Essential Oil Research, 2016. 28(2): p. 130-137. doi: 10.1080/10412905.2015.1093556
38. Francomano, F., A. Caruso, A. Barbarossa, A. Fazio, C. La Torre, J. Ceramella, et al. *β*-Caryophyllene: a sesquiterpene with countless biological properties. Applied Sciences, 2019. 9(24): p. 5420. doi: 10.3390/app9245420
39. Ayisi, N.K. and C. Nyadedzor. Comparative *in vitro* effects of AZT and extracts of *Ocimum gratissimum*, *Ficus polita*, *Clausena anisata*, *Alchornea cordifolia*, and *Elaeophorbia drupifera* against HIV-1 and HIV-2 infections. Antiviral Research, 2003. 58(1): p. 25-33. doi: 10.1016/s0166-3542(02)00166-3
40. Yucharoen, R., S. Anuchapreeda, and Y. Tragoolpua. Anti-herpes simplex virus activity of extracts from the culinary herbs *Ocimum sanctum* L., *Ocimum basilicum* L., and *Ocimum americanum* L. African Journal of Biotechnology, 2011. 10(5): p. 860-866. doi: 10.5897/AJB10.1351