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Comparative Study of *Alpinia officinarum* and *Zingiber officinale* Root Extracts for Chemical Composition, Antioxidant Potential and Cell Mediated Blocking of HSV-II Activities

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Abstract

Herpes simplex virus -2 (HSV-II) increases the prevalence of life-threatening viral infections so, there is a great need to find new compounds to combat them. This study aims to compare the chemical composition, antioxidant potential and cell-mediated blocking of HSV-II activities of *Alpinia officinarum* and *Zingiber officinale* extracts. The roots were extracted by methanol and ethyl acetate. Phenolic and flavonoid contents were determined, DPPH antioxidant activity as well as HSV-II antiviral activity. The phenolic content of ethyl acetate extract for AO and ZO (114.40 ± 8.11 and 90.42 ± 9.67 mg GAE/g dry extract, respectively) was higher than methanolic. Ethyl acetate extracts showed higher flavonoid content (299.11 ± 33.85 and 147.92 ± 24.50 mg RE/g dry extract, respectively). IC_{50} of DPPH scavenging activity was promising for AO and ZO EtOAc (23.78 and 30.03 µg/ml, respectively). The highest anti-HSV-II activity was AO EtOAc (91.9 %), followed by ZO EtOAc (50.3 %). Quantification of phenolic acid in ethyl acetate extract for plants by HPLC shows that the most prevalent phenolic compounds in ZO were Ellagic acid, Hesperetin, Querectin, Daidzein and Syringic acid (31.89, 22.91, 19.56, 18.49 and 11.33 µg/ml, respectively). while, the high content of phenolic acids in AO were Chlorogenic acid, Ellagic acid, Hesperetin, Apigenin, Syringic acid and Kaempferol (44.57, 42.05, 38.50, 29.91, 28.25 and 23.33 µg/ml, respectively). Both plants especially AO EtOAc extract were promised against HSV-II that may be returned to the presence of phenolic and flavonoid compounds.

Keywords: *Alpinia officinarium*; antioxidant potential; chemical composition; HSV-II; *Zingeber officinale*

1. Introduction

Herbal remedies have a long history of use in medicine and continue to support a wide range of health needs for people worldwide. Plants have been utilized by humans as bioresources to treat or prevent illness. This is because certain sections of them, like leaves, flowers, bark, seeds, fruits, and roots, include deposits of their active components, which include alkaloids, tannins, steroids, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids [1,2].

Zingiber officinale and *Alpinia officinarum* are members of the Zingiberaceae family. Plant species with antioxidant qualities are found in the Zingiberaceae family. The meaty rhizome and fragrant leaves help identify the majority of its species with ease. Numerous Zingiberaceae plants are utilized as flavoring agents, condiments, traditional remedies, spices, and dye sources [3].

Alpinia officinarum, also known as Galangal, is a common spice in India and other Asian nations and a rich source of many chemicals [4]. According to Liu et al. [5] it has been utilized as traditional Chinese medicine to treat colds, stomach problems, circulatory system stimulation, and edema. Antioxidant, anticancer, antiemetic, antibacterial,

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antiviral, and hypolipidemic properties have been reported for it [6,7,8,9,10].

Furthermore, for a very long time, people have used ginger, *Zingiber officinale*, as a spice and herbal remedy [11]. Its rhizomes are utilized for purposes other than just seasonings and sauces [12]. However, it also has a large number of potentially bioactive substances with both medicinal and biological properties [13]. Biological effects of ginger include antioxidant [14], anti-inflammatory [15], antimicrobial [16], antiviral [17] and anticancer [18].

Despite significant advancements in human healthcare, infectious diseases continue to be the world's greatest cause of mortality, particularly in developing nations where they take millions of lives each year [19]. One of the main causes of infectious diseases is viral infections. Because of their complexity and diversity, viral infections frequently cause pandemic events [20]. Therefore, the development of resistance to commonly used antibiotics and antiviral medications remains a serious challenge for its treatment at this time [21,22,23].

Among the viral infections is the Herpes simplex virus (HSV). It is an encapsulated DNA virus of medium size [24]. HSV-1, which causes the majority of cold sores, and HSV-2, which causes the majority of oral and genital herpes, are the two different forms of HSV infection [25,26]. About 16% of adults between the ages of 15 and 45 are infected with HSV-2, which can have a 70% fatality rate if treatment is not received [27]. Antiviral medications are therefore essential for people who have viral infections [26].

This study sought to compare the phytoconstituents of root extracts from *Zingiber officinale* and *Alpinia officinarum* plants, with an emphasis on analysing their antioxidant potential and antiviral activities against herpes simplex virus type 2 (HSV-II).

2. Experimental

2.1. Collection and preparation of extracts

Rhizomes of *Alpinia officinarum* (AO) and *Zingiber officinale* (ZO) weighing one kilogram were procured at the nearby Haraz Market for Herbs, and examined by Dr. Rim Samir Hamdy, a professor in the Plant Taxonomy Botany Department at Cairo University's Faculty of Science. Both plants' rhizomes were ground up, and 500 g of each were extracted using 85% methanol (MeOH) and the remaining 500 g were extracted over the course of seven days using ethyl acetate (EtOAc). The solvents were extracted using a rotatory evaporator (Rotavapor R 300, Buchi, Switzerland) operating under vacuum at 40°C. After being dried, each extract was stored for later research.

2.2. Total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenolic content in accordance with [28,29]. As a standard, 1 mg/ml stock solution of gallic acid was utilized in methanol. From the standard and samples, nine serial dilutions were made, namely 12.5, 25, 50, 100, 200, 400, 500, 800, and 1000 µg/ml. AO and ZO methanol and ethyl acetate extracts were made at a concentration of 5 mg/ml in methanol. The outcomes were recorded with the FluoStar Omega microplate reader. Six duplicates of each of the nine standards and samples were pipetted into the plate wells. At 630 nm, the absorbance of the total phenolic content was measured, and the average measurement from six replicates was obtained. The results were given in milligrams per gram (mg GAE/g) of gallic acid equivalents for the dry extract.

2.3. Total flavonoid content

Aluminum chloride was used to determine the total flavonoid concentration in accordance with [30]. Rutin was standardised to a stock solution of 1 mg/ml in methanol. At concentrations of (6.5, 12.5, 25, 50, 100, 200, 400, 500, 800, 1000 µg/ml), ten serial dilutions were made. Both AO and ZO methanol and ethyl acetate extracts were made at a 5 mg/ml concentration in methanol. The outcomes were recorded with the FluoStar Omega microplate reader. Six duplicates of each of the ten standards and samples were pipetted into the plate wells. At 415 nm, the absorbance of total phenolic content was measured, and the average result from six replicates was obtained. The resultant data were given as milligrams per gram of rutin equivalents (mg QE/g) of dry extract.

2.4. DPPH• radical scavenging activity

DPPH• (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to [31]. Briefly; 100µL of freshly prepared DPPH• reagent

(0.1% in methanol) was added to 100μL of the sample in 96 wells plate (n=6), and samples were prepared in final concentrations of 1000 and 100 μg/mL in MeOH. The results were recorded using a microplate reader FluoStar Omega. The reaction was incubated at room temp for 30 min in the dark. At the end of incubation time, the resulting reduction in DPPH• color intensity was measured at 540 nm. Trolox was used in different concentrations (5, 10, 15, 20, 30, 40, 50 μg/ml) as a standard. The average of six replicates are represented as means ± SD according to the following equation: Inhibition% = [(Average ABlank -Average ASample) / Average ABlank] * 100

Where: ABlank is absorbance of blank, ASample is absorbance of sample. The IC50 value represented the concentration of the samples that caused 50% inhibition and was calculated by Graph Pad Prism 5.

2.5. Determination of the maximum non-toxic concentration [MNTC] of each extract on vero cell line

From the analyzed samples, several concentrations were made. Once a confluent sheet of vero cell had grown, the growth medium was removed from 96-well microtiter plates and the cell monolayer was twice washed with wash media. The examined material was divided into twofold dilutions using DMEM. Each dilution was tested in 0.1 ml increments in several wells, with three wells serving as controls and receiving only maintenance media. For a maximum of two days, the plate was incubated at 370C and constantly checked. Physical indicators of toxicity, such as shrinkage, rounding, granulation, or partial or whole loss of the monolayer, were examined in the cells. A 5 mg/ml in PBS for MTT solution was created (BIO BASIC CANADA INC). Each well received 20 ul of MTT solution, which was then shaken vigorously at 150 rpm for five minutes to completely mix the MTT into the medium. For four hours, the plate was incubated at 370C with 5% CO2 to facilitate the metabolism of MTT. The plate was left to dry once the media were removed. After being resuspended in formazan (a MTT metabolic product) in 200 ul of DMSO, the wells were shaken for five minutes at 150 rpm on a shaking table. Background at 620 nm was eliminated from the optical density obtained at 560 nm. There should be a direct relationship between cell amount and optical density. Each extract's maximum non-toxic concentration (MNTC) was calculated, and the results were applied to the following additional biological investigations.

2.6. HPLC identification and quantification of phenolic compounds

Phenolic and flavonoid component analyses were carried out with HPLC equipment (Agilent 1260 series). The Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm) was used for the analysis. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, which was flowing at a rate of 0.9 ml/min. The following was the sequential linear gradient programming for the mobile phase: 8–12 min (60% A), 0–5 min (80% A), 12–15 min (82% A), 15–16 min (82% A), and 16–20 min (82% A). At 280 nm, the multi-wavelength detector was observed. For every sample solution, there was one injection volume of five microliters. At 40 °C, the column temperature was kept constant. Standard phenolic acids were prepared at a concentration of 10 mg/50 ml in methanol and diluted to 20–40 μg/ml. Peak area computation (external standard method) was used to quantify the primary components found in the plant extract.

3. Results and Discussion

3.1. Total Phenolic content

Since hydroxyl groups scavenge free radicals, phenolic chemicals are attractive secondary components with redox characteristics. The Folin-Ciocalteu reagent was used in this investigation to measure the total phenolic content. The results were reported in gallic acid equivalents (GAE) per gram dry extract weight and were obtained using a calibration curve of gallic acid (12.5-1000 μg/ml) (y = 0.0036x − 0.1174, R2 = 0.9917) (Fig 1). In comparison to the methanolic extracts of both plants (80.20 ± 6.18 and 65.14 ± 7.36 mg GAE/g dry extract, respectively), the phenolic content in the ethyl acetate extracts for AO and ZO (114.40 ± 8.11 and 90.42 ± 9.67 mg GAE/g dry extract, respectively) was higher. In contrast to earlier findings in the literature, ZO's total phenolic content for ethanol and methanol extracts was found to be in full agreement with our results [32,13]. Also, our results of AO were in concise with [33,34]. According to Burri et al. [35], there may be variations in phenolic content values between studies based on factors such as duration, extraction process, and regional variation. In actuality, the total phenolic content of the AO and ZO EtOAc extracts was higher than that of the other

extracts. Despite that they are all related to one family.

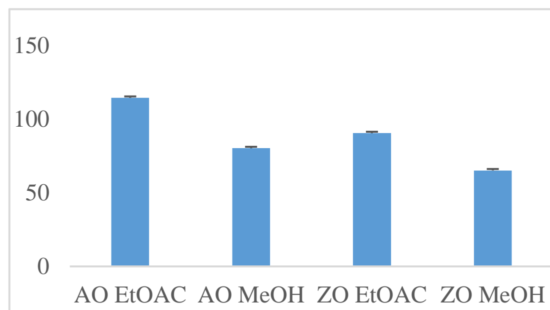


Fig 1: Total phenolic content of AO and ZO extracts

3.2. Total Flavonoid content

This study used a colorimetric technique using aluminum chloride to assess the flavonoid concentrations of certain plant extracts (Fig 2). Rutin (6.25-1000 µg/ml) was used as the calibration curve for the results, which were expressed in rutin acid equivalents (RE) per gram dry extract weight (y = 0.0014x + 0.0660, R2 = 0.9986). In both plants, total flavonoid level was promising; ethyl acetate extract had higher flavonoid content than methanolic (286.57 ± 15.39 and 8.96 ± 6.06 mg RE/g dry extract, respectively for AO), and (299.11 ± 33.85 and 147.92 ± 24.50 mg RE/g dry extract, respectively for ZO). The quantity of free OH groups determines the antioxidant potency of flavonoids [36]. ZO total flavonoid content was measured by Tohma et al. [13] at 25.1% dry extract, whereas, AO total flavonoid content was measured by Malik et al. [34] at 51.76 ± 1.28 mg RE/g dry extract. Plants flavonoid concentration was found to be highly biological impact [37]. Our research suggests that AO contains more flavonoids than phenols, which could be related to solvents' propensity to extract flavonoids more readily than phenols [38].

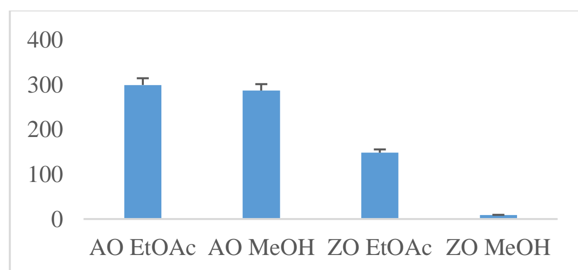
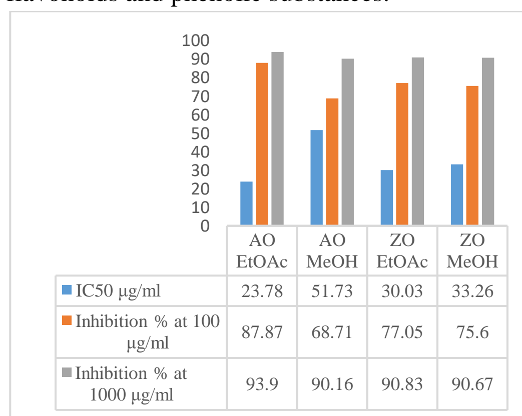


Fig 2: Total flavonoid content of AO and ZO extracts

3.3. DPPH• radical scavenging activity

This assay is quick, simple, and affordable, DPPH• is frequently used in laboratories to assess the capacity of phenolic compounds and natural plant extracts to scavenge free radicals [39]. The inhibition % and DPPH• IC50 of the AO and ZO extracts were displayed in (Fig.3). The findings showed that for the two studied plants, EtOAc extracts had a 50% greater capacity for scavenging than MeOH extracts. The DPPH• assay quantifies a compound's capacity to donate hydrogen or scavenge free radicals [40,41]. Our findings corroborated other studies' findings that AO's various extracts had DPPH• scavenging capability [42,43] and Additionally, in line with the findings of Stoilova et al. [32] and Mošovská et al. [44], who demonstrated the ability of several extracts of ZO to scavenge DPPH free radicals. Both plants' scavenging capacity may be attributed to the presence of flavonoids and phenolic substances.



	AO EtOAc	AO MeOH	ZO EtOAc	ZO MeOH
IC50 µg/ml	23.78	51.73	30.03	33.26
Inhibition % at 100 µg/ml	87.87	68.71	77.05	75.6
Inhibition % at 1000 µg/ml	93.9	90.16	90.83	90.67

Fig. 3: DPPH scavenging activity of AO and ZO extracts

3.4. Determination of the maximum non-toxic concentration [MNTC] of each extract on Vero cell line

The maximum non-toxic concentration (MNTC) for each plant extract was ascertained by testing its cytotoxicity against Vero cells. The outcomes showed that 100% of normal Vero cells were viable. MNTC was found in extracts AO EtOAc, ZO EtOAc, and ZO MeOH at 31.25 µg/ml. In contrast, MNTC was 62.5 µg/ml in AO MeOH. Additionally, their cell viability percentages (98.19%, 98.62%, 96.46%, and 100%, respectively) resembled those of normal Vero cells. On the other hand, Fig. (4 &5) mentioned MNTC and IC50. In fact, our findings showed that neither of the two plant extracts had any harmful effects. Furthermore, AO MeOH resembled regular

cells. These findings, along with earlier research, demonstrated that AO and ZO plants are not harmful to Vero cells [45,46,47,48].

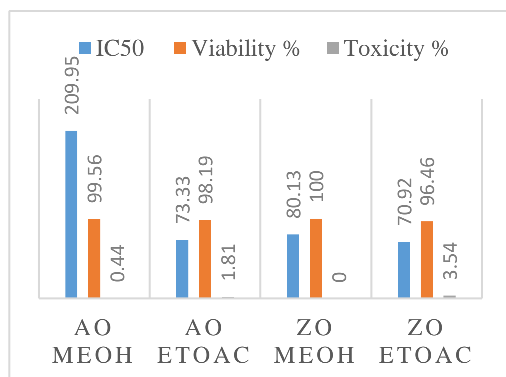


Fig (4): Effect of AO and ZO extracts maximum non-toxic concentration (MNTC) on vero cell line

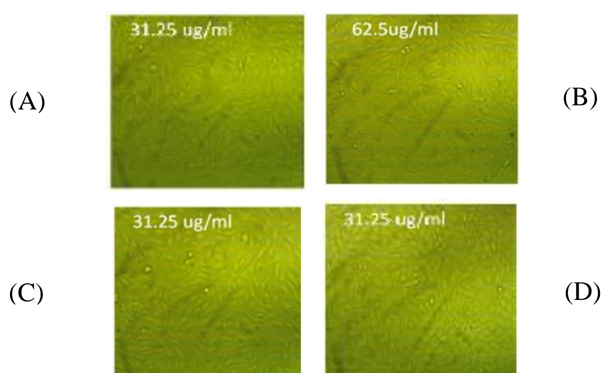


Fig. (5): Effect of different fractions on vero cells at different concentrations (1000, 500, 250, 125, 62.5 and 31.25 ug/ml) shows (A) MNTC of AO EtOAc to be 31.25 ug/ml. (B) MNTC of AO MeOH to be 62.5 ug/ml, (C) MNTC of ZO EtoAc to be 31.25 ug/ml, (D) MNTC of ZO MeOH to be 31.25 ug/ml.

3.5. Antiviral assay dependent MNTC against Herpes simplex type-II (HSV-II)

The following diagram (Fig.6) illustrates how antiviral assay-dependent MNTC against Herpes simplex type-II (HSV-II) exhibits dramatically variable activity %. At a dosage of 31.25 ug/ml, AO EtOAc had the strongest anti-HSV-II (91.9 %) antiviral activity and the lowest toxicity (3.69 %), followed by ZO EtOAc (50.3 %) antiviral and toxicity (22.9%). ZO MeOH also demonstrated anti-HSV II action (42.34%) and toxicity (26.53%). The AO MeOH extract showed the least amount of action. In actuality, this is the first report on Zingiber officinale and Alpinia officinarum's anti-HSV-II activity. But according to Ramadan [49], ZO essential oil is a viruscidal agent against HSV-II. The combined phenolic and flavonoid contents of the two plants could be the cause of such. However, these results indicate that the phenolic components of the extracts have antiviral activity against the Herpes simplex virus type 2.

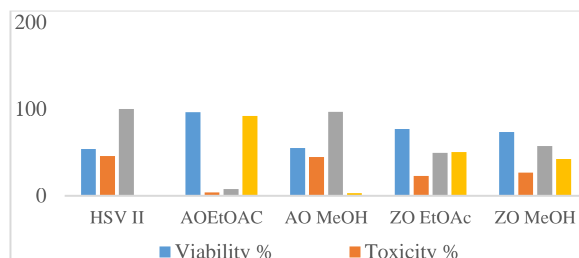


Fig. 6: Antiviral activity of AO and ZO extracts against Herpes simplex type II revealed antiviral significant percentage.

3.6. HPLC identification and quantification of phenolic compounds:

The EtOAc extracts of AO and ZO in this study have a high concentration of phenolic components, which may produce antioxidant and antiviral activities, according to the results of the total phenolic and flavonoid content assay. Therefore, using HPLC analyses to determine the chemical composition and relative proportions of phenolic compounds in the extracts, the EtOAc extracts of plants were compared to nineteen standard phenolic compounds (Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Syringic acid, Pyro catechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Daidzein, Querectin, Cinnamic acid, Apigenin, Kaempferol, and Hesperetin), as shown in Tables (1), Fig. (7).

The most prevalent phenolic acids in ZO extract were Ellagic acid, Hesperetin, Querectin, Daidzein and Syringic acid (31.89, 22.91, 19.56, 18.49 and 11.33 µg/ml, respectively). The other chemicals were detected in trace levels, whereas pyrocatechol and rutin were absent.

Chlorogenic acid, Ellagic acid, Hesperetin, Apigenin, Syringic acid, and Kaempferol were the most common phenolic acids in AO extract (44.57, 42.05, 38.50, 29.91, 28.25, and 23.33 µg/ml, respectively). Rutin, Pyrocatechol, Catechin, and Coffeic acid were absent, and the concentration of the

other components in this extract was greater than that of the ZO extract.

Despite coming from the same family, the two plants differ greatly in terms of their levels of comparable chemicals and their chemical composition. Therefore, AO may be more active than ZO as a result of these changes [50].

Three phenolic acids; elagic acid, syringic acid, and chlorogenic acid; are beneficial to health because they may serve as antioxidants to prevent oxidative stress on cells brought on by free radical oxidation processes [51]. In AO, elagic acid and syringic acid are more concentrated than in ZO. Ellagic acid demonstrated increased antiviral efficacy and partially inhibited the replication of HSV-II by targeting cellular molecules [52,53,54]. it has been reported that AO contains chlorogenic acid, which has demonstrated antiviral properties against several virus species, including Herpes Simplex Virus-1 (HSV-I) and Herpes Simplex Virus-2 (HSV-II) [55,56].

Flavonoids, including hesperetin, querectin, daidzein, apigenin, and kaempferol, offer a range of possible biological advantages, including anti-inflammatory, antioxidant, antibacterial, anticancer, antifungal, and antiviral properties [57].

Whereas querectin and daidzein are more abundant in ZO extract than AO extract, apigenin, kaempferol, and hesperetin are more abundant in Ao extract. Hesperetin content in AO is over twice that of ZO extract. Hesperetin has demonstrated antiviral effectiveness against a variety of viruses, including COVID-19 and HSV-1, through reducing the virus's intracellular replication, according to earlier research [58,57,59,60]. Furthermore, it can prevent HSV-II plaques from forming [61]. The high concentration of apigenin and kaempferol found specifically in AO extract may account for its potent antiviral properties. According to [57], apigenin demonstrates a range of antiviral properties against many viruses, including poliovirus type 2, hepatitis C, and B viruses. By preventing infection at the viral post-entry stage, it was also discovered to be effective against HSV-I and HSV-II [62,63]. Due to its antiviral, antibacterial, antioxidant, anticancer, and anti-inflammatory properties, kaempferol has drawn a lot of interest [64]. Kaempferol inhibits the replication of HSV-II, demonstrating strong anti-HSV-II activity, according to several researches [65,66,57].

ZO extract has a moderate content of querectin. There have been reports that querectin reduces the infectivity of viruses in a concentration-dependent manner. Furthermore, it decreased the in-vitro virus's (HSV-I and II) intracellular replication [58,66,67]. Several investigations have demonstrated that daidzein has no antiviral impact against the herpes simplex virus (HSV), despite the fact that it is found in ZO extract in a modest proportion [68,69].

These data collectively demonstrate that ethyl acetate extracts of AO and ZO have a high concentration of phenolic components that possess the ability to scavenge free radicals and may help prevent the intracellular reproduction and infection of Herpes Simplex Virus-II (HSV-II).

Table (1): Comparative Phenolic acid concentrations in EtOAc of ZO and AO extracts compared to standard phenolic compounds

Compounds	Rt	Standard Conc. (µg/ml)	EtOAc of ZO Conc.(µg/ml)	EtOAc of AO Conc.(µg/ml)
Gallic acid	3.43	15	5.84	3.31
Chlorogeni acid	4.23	50	7.39	44.57
Catechin	4.64	75	6.21	-------
Methyl gallate	5.63	15	1.36	10.86
Coffeic acid	6.08	18	3.31	-------
Syringic acid	6.59	17.2	11.33	28.25
Pyro catechol	6.84	40	-------	-------
Rutin	7.90	61	-------	--------
Ellagic acid	8.78	120	31.89	42.05
Coumaric acid	9.17	20	0.45	2.64
Vanillin	9.79	12.9	8.85	13.72
Ferulic acid	10.26	20	6.46	6.31
Naringenin	10.48	30	4.89	7.50
Daidzein	12.26	35	18.49	7.10
Querectin	12.74	40	19.56	1.65
Cinnamic acid	14.04	10	4.54	2.84
Apigenin	14.48	50	1.48	29.91
Kaempferol	14.98	60	13.61	23.33
Hesperetin	15.54	20	22.91	38.50

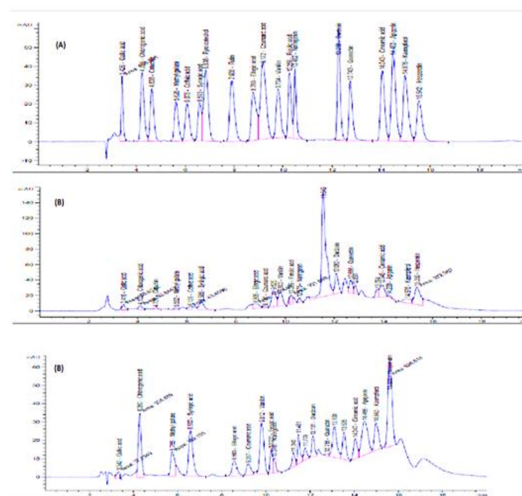


Fig (7): HPLC-chromatogram of (A): standards phenolic compounds, (B): EtOAc of ZO and (C): EtOAc of AO

4. Conclusion

The ethyl acetate extracts of *A. officinarum* and *Z. officinale* have a high content of phenolic components, which may be the main cause of antioxidant activity and inhibit the intracellular replication and infection of Herpes Simplex Virus-II (HSV-II), according to the results of the current study's total phenolic, flavonoid, and HPLC content assays.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

This study did not receive any fund or grants.

7. Credit authorship contribution statement

Heba Abdel-Hady: conceptualization, data curation, methodology, formal analysis, validation, writing - original draft. Manal Mortada Hamed: visualization, data curation, methodology validation, writing - original draft. Sami Mohamed Nasr: conceptualization, data curation, methodology, formal analysis, validation, writing - original draft. Eman Ahmed El-wakil: Visualization, Validation, Writing- original draft. Eman Abdallah Morsi: conceptualization, data curation, validation, writing - original draft. All authors share writing - reviewing and editing.

8. References

[1] Hamed, M.M., (2007). Volatile oils from *Pelargonium zonale* and its cytotoxic effect on tumor cells. *Bull. Fac. Pharm. Cairo University*. 45 (3): 297-300.

[2] Chandrakar, A. K. (2018). Phytochemical fingerprinting through computerized HPTLC System for quality control of herbal drugs. International Journal for Research in Applied Science and Engineering Technology. 6(733): 2321-9653.

[3] Akinola, A. A., Ahmad, S., Maziah, M. (2014). Total anti-oxidant capacity, flavonoid, phenolic acid and polyphenol content in ten selected species of Zingiberaceae rhizomes. African Journal of Traditional. Complementary and Alternative Medicines. 11(3): 7-13.

[4] Ma, X. N., Lan Xie, C.L., Miao, Z, Yang, Q. and Yang, X. W. (2017). An overview of chemical constituents from *Alpinia species* in the last six decades. RSC Adv 7, 14114–14144.

[5] Liu, D., Liu, Y. W., Guan, F. Q., & Liang, J. Y. (2014). New cytotoxic diarylheptanoids from the rhizomes of *Alpinia officinarum* Hance. Fitoterapia. 96: 76-80.

[6] An, N., Zou, Z.M., Tian, Z., Luo, X.Z., Yang, S.L. and Xu, L.Z. (2007). Diarylheptanoids from the rhizomes of *Alpinia officinarum* and their anticancer activity. Fitoterapia. 79: 27–31.

[7] Shin, D., Kinoshita, K., Koyama, K. and Takahashi, K. (2002). Antiemetic principles of *Alpinia officinarum*. J Nat Prod. 65: 1315–1318

[8] Zhang, B.-B., Dai, Y., Liao, Z.-X. and Ding, L.-S. (2010). Three new antibacterial active diarylheptanoids from *Alpinia officinarum*. Fitoterapia. 81: 948–952.

[9] Honmore, V.S., Rojatkar, S.R., Nawale, L.U., Arkile, M.A., Khedkar, V.M., Natu, A.D. and Sarkar, D. (2016). In vitro and ex vivo antitubercular activity of diarylheptanoids from the rhizomes of Alpinia officinarum Hance. Nat Prod Res. 30:2825–2830.

[10] Xia, D.Z., Yu, X.F., Wang, H.M., Ren, Q.Y. and Chen, B.M. (2010). Anti-obesity and hypolipidemic effects of ethanolic extract from *Alpinia officinarum* Hance (Zingiberaceae) in rats fed high-fat diet. J Med Food. 13: 785–791.

[11] Han, Y.A., Song, C.W., Koh, W.S., Yon, G.H., Kim, Y.S., Ryu, S.Y., Kwon, H.J. and Lee, K.H. (2013). Anti-inflammatory effects of the Zingiber officinale roscoe constituent 12-dehydrogingerdione in lipopolysaccharide-stimulated Raw 264.7 cells. Phytotherapy Research. *27*(8):1200-1205.

[12] Baliga, M. S., Haniadka, R., Pereira, M. M., D'Souza, J. J., Pallaty, P. L., Bhat, H. P., Popuri, S. (2011). Update on the chemopreventive effects of ginger and its phytochemicals. Critical reviews in food science and nutrition, 51(6): 499-523.

[13] Tohma, H., Gülçin, İ., Bursal, E., Gören, A. C., Alwasel, S. H., & Köksal, E. (2017). Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. Journal of food measurement and characterization. 11(2): 556-566.

[14] Nile, S. H., & Park, S. W. (2015). Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. Industrial Crops and Products. 70: 238-244.

[15] Zhang, M., Viennois, E., Prasad, M., Zhang, Y., Wang, L., Zhang, Z., Han, M.K., Xiao, B., Xu, C., Srinivasan, S. and Merlin, D. (2016). Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. Biomaterials. 101:321-340.

[16] Kumar, N. V., Murthy, P. S., Manjunatha, J. R., Bettadaiah, B. K. (2014). Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. Food Chemistry. 159: 451-457.

[17] Dissanayake, K. G. C., Waliwita, W. A. L. C., Liyanage, R. P. (2020). A review on medicinal uses of *Zingiber officinale* (ginger). International Journal of Health Sciences and Research. 10(6): 142-148.

[18] Citronberg, J., Bostick, R., Ahearn, T., Turgeon, D. K., Ruffin, M. T., Djuric, Z., Sen, A., Brenner, D.E & Zick, S. M. (2013). Effects of ginger supplementation on cell-cycle biomarkers in the normal-appearing colonic mucosa of patients at increased risk for colorectal cancer: results from a pilot, randomized, and controlled trial. Cancer Prevention Research. 6(4): 271-281.

[19] York, T., De Wet, H., & Van Vuuren, S. F. (2011). Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. Journal of ethnopharmacology. 135(3): 696-710.

[20] Denaro, M., Smeriglio, A., Barreca, D., De Francesco, C., Occhiuto, C., Milano, G., & Trombetta, D. (2020). Antiviral activity of plants and their isolated bioactive compounds: An update. Phytotherapy Research. 34(4): 742-768.

[21] Morens, D. M., Folkers, G. K., & Fauci, A. S. (2004). The challenge of emerging and re-emerging infectious diseases. Nature. 430 (6996): 242-249.

[22] de Souza, M. V. N. (2009). Promising candidates in clinical trials against multidrug-resistant tuberculosis (MDR-TB) based on natural products. Fitoterapia. 80(8): 453-460.

[23] Chen, Y. L., Huang, S. T., Sun, F. M., Chiang, Y. L., Chiang, C. J., Tsai, C. M., & Weng, C. J. (2011). Transformation of cinnamic acid from trans-to cis-form raises a notable bactericidal and synergistic activity against multiple-drug resistant Mycobacterium tuberculosis. European Journal of Pharmaceutical Sciences. *43*(3): 188-194.

[24] Kaufman, H. E., Azcuy, A. M., Varnell, E. D., Sloop, G. D., Thompson, H. W., & Hill, J. M. (2005). HSV-1 DNA in tears and saliva of normal adults. Investigative Ophthalmology & Visual Science. 46(1): 241-247.

[25] Agelidis, A. M., Shukla, D. (2015). Cell entry mechanisms of HSV: what we have learned in recent years. Future virology. 10(10): 1145-1154.

[26] Du, Q., Gu, Z., Leneva, I., Jiang, H., Li, R., Deng, L., & Yang, Z. (2019). The antiviral activity of arbidol hydrochloride against herpes simplex virus type II (HSV-2) in a mouse model of vaginitis. International Immunopharmacology. 68: 58-67.

[27] Looker, K. J., Garnett, G. P., & Schmid, G. P. (2008). An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bulletin of the World Health Organization. 86(10): 805-812A.

[28] Attard, E. (2013). A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols. Open Life Sciences. 8(1): 48-53.

[29] Hamed, M.M., Abdalla, A.M., Ghareeb, M.A., Said Abdelhalim Saleh S.A. (2017). Chemical constituents, *in vitro* antioxidant activity, oral

acute toxicity and LD_{50} determination of *Moringa oleifera* leaves. International Journal of Pharmacy and Pharmaceutical sciences. 9 (5): 240-247.

[30] Kiranmai, M., Kumar, C. M., Mohammed, I. (2011). Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. Research Journal of Pharmaceutical, Biological and Chemical Sciences. *2*(3): 254-261.

[31] Boly R, Lamkami T, Lompo M, Dubois J, Guissou I. (2016). DPPH Free Radical Scavenging Activity of Two Extracts from *Agelanthus dodoneifolius* (Loranthaceae) Leaves. International Journal of Toxicological and Pharmacological Research. 8(1): 29-34.

[32] Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P., & Gargova, S. (2007). Antioxidant activity of a ginger extract (Zingiber officinale). Food chemistry: 102(3): 764-770.

[33] Srividya, A. R., Dhanabal, S. P., Misra, V. K., & Suja, G. (2010). Antioxidant and antimicrobial activity of Alpinia officinarum. Indian journal of pharmaceutical sciences. *72*(1): 145.

[34] Malik, T., Pandey, D. K., Roy, P., & Okram, A. (2016). Evaluation of phytochemicals, antioxidant, antibacterial and antidiabetic potential of *Alpinia galanga* and *Eryngium foetidum* plants of Manipur (India). Pharmacognosy Journal. 8(5).

[35] Burri, S. C., Ekholm, A., Håkansson, A., Tornberg, E., Rumpunen, K. (2017). Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. Journal of functional foods. 38:119-127.

[36] Panche, A. N., Diwan, A. D., Chandra, S. R. (2016). Flavonoids: an overview. Journal of Nutritional Science, 5.

[37] Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 8(4): 96.

[38] Adaramola, B., Onigbinde, A. (2016). Effect of extraction solvent on the phenolic content, flavonoid content and antioxidant capacity of clove bud. IOSR J Pharm Biol *Sci*, 11(3):33-8.

[39] Shalaby, E.A., Shanab, S.M.M. (2013). Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis. Indian J Mar Sci. 42(5):556-564.

[40] Kedare SB, Singh RP. (2011). Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 48(4):412-422.

[41] El-Sayed, M. M., Hashash, M. M., Abdel-Hady, A. A., Abdel-Hady, H., Abdel-Lateef, E. E., & Morsi, E. A. (2017). Total phenolic and flavonoid contents and antioxidant activity of *Lantana camara* and *Cucurbita pepo* (Squash) extracts as well as GC-MS analysis of *Lantana camara* essential oils. *World J Pharm Res*. *6*(1): 137-153.

[42] Fang, L., Zhang, H., Zhou, J., Geng, Y., & Wang, X. (2018). Rapid screening and preparative isolation of antioxidants from Alpinia Officinarum Hance using HSCCC coupled with DPPH-HPLC assay and evaluation of their antioxidant activities. Journal of Analytical Methods in Chemistry. 2018.

[43] Zhang, W.X., Chao, I.C., Hu, D.J., Shakerian, F., Ge, L., Liang, X., Wang, Y., Zhao, J. and Li, S.P. (2019). Comparison of antioxidant activity and main active compounds among different parts of Alpinia officinarum Hance using high-performance thin layer chromatography-bioautography. Journal of AOAC International. 102(3):726-733.

[44] Mošovská, S., Nováková, D., & Kaliňák, M. (2015). Antioxidant activity of ginger extract and identification of its active components. Acta Chimica Slovaca. 8(2): 115-119.

[45] Konno, K., Sawamura, R., Sun, Y., Yasukawa, K., Shimizu, T., Watanabe, W., Kato, M., Yamamoto, R. and Kurokawa, M. (2011). Antiviral activities of diarylheptanoids isolated from Alpinia officinarum against respiratory syncytial virus, poliovirus, measles virus, and herpes simplex virus type 1 in vitro. Natural product communications. 6(12): 1934578X1100601222.

[46] Jeena, K., Liju, V. B., Kuttan, R. (2015). Antitumor and cytotoxic activity of ginger essential oil (*Zingiber officinale* Roscoe). Int J Pharm Pharm sci, *7*(8): 341-344.

[47] Ahmed, I., Aslam, A., Mustafa, G., Masood, S., Ali, M. A., & Nawaz, M. (2017). Anti-avian influenza virus H9N2 activity of aqueous extracts of *Zingiber officinalis* (Ginger) and *Allium sativum* (Garlic) in chick embryos. Pak. J. Pharm. Sci. 30(4): 1341-1344.

[48] Abubakar, I. B., Malami, I., Yahaya, Y., & Sule, S. M. (2018). A review on the ethnomedicinal uses, phytochemistry and pharmacology of

Alpinia officinarum Hance. Journal of Ethnopharmacology. *224*:45-62.

[49] Ramadan, M. F. (2020). Cold pressed ginger (*Zingiber officinale*) oil. In *Cold pressed oils* (pp. 677-682). Academic Press.

[50] Basri, A. M., Taha, H., Ahmad, N. (2017). A review on the pharmacological activities and phytochemicals of *Alpinia officinarum* (Galangal) extracts derived from bioassay-guided fractionation and isolation. Pharmacognosy Reviews. 11(21): 43.

[51] Kumar, N., Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports. 24: e00370.

[52] Park, S. W., Kwon, M. J., Yoo, J. Y., Choi, H. J., & Ahn, Y. J. (2014). Antiviral activity and possible mode of action of ellagic acid identified in Lagerstroemia speciosa leaves toward human rhinoviruses. BMC complementary and alternative medicine. 14(1): 1-8.

[53] Arunkumar, J., & Rajarajan, S. (2018). Study on antiviral activities, drug-likeness and molecular docking of bioactive compounds of *Punica granatum* L. to Herpes simplex virus-2 (HSV-2). Microbial Pathogenesis. 118: 301-309.

[54] Sharifi-Rad, J., Quispe, C., Castillo, C. M. S., Caroca, R., Lazo-Vélez, M. A., Antonyak, H., ... & Cho, W. C. (2022). Ellagic Acid: A Review on Its Natural Sources, Chemical Stability, and Therapeutic Potential. Oxidative Medicine and Cellular Longevity. 2022.

[55] Chiang, L. C., Chiang, W., Chang, M. Y., Ng, L. T., & Lin, C. C. (2002). Antiviral activity of Plantago major extracts and related compounds in vitro. Antiviral research. 55(1): 53-62.

[56] Adeosun, W. B., More, G. K., Steenkamp, P., & Prinsloo, G. (2022). Influence of seasonal and geographic variation on the anti-HSV-1 properties and chlorogenic acids content of Helichrysum aureonitens Sch. Bip. Frontiers in Molecular Biosciences. 9:961859-961859.

[57] Zakaryan, H., Arabyan, E., Oo, A., & Zandi, K. (2017). Flavonoids: promising natural compounds against viral infections. Archives of virology. 162(9): 2539-2551.

[58] Kaul, T. N., Middleton Jr, E., Ogra, P. L. (1985). Antiviral effect of flavonoids on human viruses. Journal of medical virology. 15(1): 71-79.

[59] Lee, J. H., Eo, S. K., Kim, Y. S., Lee, C. K., Han, S. S. (1999a). Antiherpetic activities of natural hesperetin alone and in combinations with acyclovir and vidarabine. *Korean* Journal of Pharmacognosy. *30*(1): 40-47.

[60] Arjsri, P., Srisawad, K., Mapoung, S., Semmarath, W., Thippraphan, P., Umsumarng, S., Yodkeeree, S., Dejkriengkraikul, P. (2022). Hesperetin from Root Extract of Clerodendrum petasites S. Moore Inhibits SARS-CoV-2 Spike Protein S1 Subunit-Induced NLRP3 Inflammasome in A549 Lung Cells via Modulation of the Akt/MAPK/AP-1 Pathway. International Journal of Molecular Sciences. 23(18): 10346.

[61] Lee, J. H., Kim, Y. S., Lee, C. K., Lee, H. K., Han, S. S. (1999b). Antiviral activity of some flavonoids on herpes simplex viruses. Korean Journal of Pharmacognosy, 30(1): 34-39.

[62] El-Toumy, S. A., Salib, J. Y., El-Kashak, W. A., Marty, C., Bedoux, G., Bourgougnon, N. (2018). Antiviral effect of polyphenol rich plant extracts on herpes simplex virus type 1. Food Science and Human Wellness. 7(1): 91-101.

[63] Rittà, M., Marengo, A., Civra, A., Lembo, D., Cagliero, C., Kant, K., Lal, U.R., Rubiolo, P., Ghosh, M. and Donalisio, M. (2020). Antiviral activity of a *Arisaema tortuosum* leaf extract and some of its constituents against herpes simplex virus type 2. Planta Medica. 86(04): 267-275.

[64] Li, L., Wang, R., Hu, H., Chen, X., Yin, Z., Liang, X., He, C., Yin, L., Ye, G., Zou, Y. and Yue, G. (2021). The antiviral activity of kaempferol against pseudorabies virus in mice. BMC Veterinary Research. *17*: 1-14.

[65] Behbahani, M., Sayedipour, S., Pourazar, A., & Shanehsazzadeh, M. (2014). In vitro anti-HIV-1 activities of kaempferol and kaempferol-7-O-glucoside isolated from *Securigera securidaca*. Research in pharmaceutical sciences. 9(6): 463.

[66] Ürményi, F. G. G., Saraiva, G. D. N., Casanova, L. M., Matos, A. D. S., de Magalhaes Camargo, L. M., Romanos, M. T. V., & Costa, S. S. (2016). Anti-HSV-1 and HSV-2 flavonoids and a new Kaempferol triglycoside from the medicinal plant Kalanchoe daigremontiana. Chemistry & Biodiversity. 13(12): 1707-1714.

[67] Lee, S., Lee, H. H., Shin, Y. S., Kang, H., Cho, H. (2017). The anti-HSV-1 effect of quercetin is

dependent on the suppression of TLR-3 in Raw 264.7 cells. Archives of Pharmacal Research. 40: 623-630.

[68] Zandi, K., Teoh, B. T., Sam, S. S., Wong, P. F., Mustafa, M. R., & AbuBakar, S. (2011). Antiviral activity of four types of bioflavonoid against dengue virus type-2. Virology journal. 8(1): 1-11.

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