A Comparative Analysis of the Bioactive Properties of Cumin and Musk monkeyflower Ethanolic Extracts

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Yasser S. Helmy[1*], Fatema Adel[2], Ahmed M. Aboul Enein[1], Yousef Khaled[2], Emad A. Shalaby[1], Ingy M. El Hefny[2], and Layla S. Tawfeek[1]

[1]Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt.
[2]Faculty of Biotechnology, October University for Modern Science and Arts (MSA), Giza, Egypt.
* The corresponding author e-mail address: yshelmy@cu.edu.eg

ABSTRACT

Plant secondary metabolites are bioactive compounds with antioxidant, antimicrobial, and antiviral effects, offering potential in preventing oxidative stress-related diseases like cancer, neurodegeneration, and chronic inflammation. The study investigated the chemical composition and biological activity of ethanol extracts from Cuminum cyminum (cumin) and Erythranthe moschata (musk monkeyflower), highlighting their therapeutic potential. The study analyzed the extracts for their content of phenols, flavonoids, condensed tannins, and ascorbic acid, using HPLC to identify specific phenolic compounds. The antioxidant capacity was evaluated through multiple assays, including DPPH, ABTS, $KMnO_4$, H_2O_2. Antibacterial activity was assessed by the agar well diffusion method, and antiviral activity was evaluated using the MTT assay on Vero E6 cells infected with human coronavirus 229E. Results showed that cumin extract contained higher levels of phenolics and flavonoids, contributing to its stronger antioxidant activity, whereas musk extract had elevated ascorbic acid content and demonstrated moderate antiviral effects. Both extracts exhibited antimicrobial properties, indicating their potential as sources of bioactive compounds for further investigation in the context of oxidative stress-related diseases. These findings highlight the therapeutic promise of plant-derived metabolites and support the need for further pharmacological investigation.

Key words: *Cuminum cyminum (cumin), Erythranthe moschata (musk monkeyflower), phytochemical screening, biological activities*

INTRODUCTION

Plants have long been used as a source of medicine; thanks to the various bioactive molecules they produce during metabolism. These phytochemicals have been shown to have various health benefits, including anti-inflammatory, antidiarrheal, antimicrobial, antioxidant, and antiviral. The structure and chemical composition of these compounds determine their therapeutic potential (1,2).

Spices are dried aromatic plant parts, such as buds, roots, or fruits, used to flavors food. They are not considered significant sources of nutrition as defined by the Food and Drug Administration (FDA). They have been used for centuries for their flavors and health benefits. However, they contain bioactive compounds, such as essential oils, antioxidants, and vitamins, with many potential health benefits. As our lifestyles have changed and we have started eating more processed foods, the use of spices has also increased (3,4).

Cumin (*Cuminum cyminum* L.), an aromatic plant from the *Apiaceae* family, is characterized by its branched stem, lobed leaves, small flowers, and spindle-shaped fruits. Its seeds are brown, fragrant, and pungent. Cumin thrives in semi-arid climates across many countries and is one of the most widely cultivated herbs globally, second only to black pepper. With its strong aroma and bitter taste, cumin has a long history of use in both traditional medicine and industry. Cumin seeds are rich in a diverse beneficial phytochemical, including terpenes, phenols, alcohols, aldehydes, flavonoids, alkaloids, coumarins, anthraquinones, saponins, tannins, steroids, proteins, resins, vitamins, and minerals. They also contain phenolic acids such as gallic, cinnamic, rosmarinic, coumaric, and vanillic acids. Cumin seeds have demonstrated antimicrobial activity against various microorganisms and are considered antioxidants due to their high phenolic content (5–10).

Musk monkeyflower (*Erythranthe moschata*, hereafter mentioned as musk) belongs to the Phrymaceae family. It is a perennial herb with a musky scent and can vary in appearance. It has upright stems, oval-shaped leaves, and yellow tubular flowers. Musk is native to North America but has been introduced to other continents. Musk plants contain many different phytochemicals, including macrocyclic ketones, pyridine, steroids, fatty acids, amino acids, peptides, and proteins. Macrocyclic ketones, steroids, and some peptides are the main active ingredients in musk. Muscone, the main active ingredient in musk, has several pharmacological effects, including anti-inflammatory, antimicrobial, anticancer, neuroprotective, and cardiovascular-protective activities. It can also induce liver drug metabolism enzymes. These bioactive compounds are what make musk useful in traditional medicine. Additionally, musk has been used in the perfume industry for hundreds of years in Europe (11,12).

This study investigated the phenolic profile and biological properties of ethanolic extracts and semi-purified fractions from *E. moschata* and *C. cyminum*.

MATERIALS AND METHODS

Chemicals and reagents

Hexane, ethanol, ethyl acetate and methanol were purchased from E. Merck Co. (Darmstadt, Germany). Sulforhodamine B, 2,2 diphenyl-1-picrylhydrazyl (DPPH), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2), vanillin, 2, 6-dichlorophenolindophenol (DCPIP), K_2HPO_4, NaH_2PO_4 and Ascorbic acid were purchased from Sigma-Aldrich Company for Chemicals (St. Louis, MO, USA). All solvents were HPLC grade and redistilled before use.

Plant Samples and Extraction

The *C. cyminum* and *E. moschata* plants were collected from the local market in Giza, Egypt during the fall of 2022. Professor Sanaa Shanab of the Botany Department at Cairo University authenticated the collected biomass (personal communication). After air-drying, the biomass was ground into a fine powder. To prepare the extract, 25 grams of the dried biomass powder was mixed with 70% ethanol and stirred continuously, then filtered through Whatman No. 1 filter paper. The extract was dried at 40°C using a rotary evaporator (Maubon, USA) and stored at -5°C.

Qualitative phytochemical screening of ethanolic extract

Musk and cumin ethanolic extracts were tested for the presence of major phytochemicals, including phenols, flavonoids, alkaloids, anthocyanins, and coumarins, using standard methods (13). The phenolic content was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents per gram of dry weight. Total flavonoids were quantified using the aluminum chloride colorimetric method and expressed as quercetin equivalents per gram of dry weight. Condensed tannins were estimated using the vanillin method and expressed as catechin equivalents per gram of dry weight. Ascorbic acid was determined by titration with 2,6-dichlorophenolindophenol (DCPIP) and expressed as ascorbic acid equivalents per milliliter of extract (14–18).

HPLC Quantitative analysis of phenolic compounds in musk and cumin extracts

High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1260 series system equipped with a multi-wavelength detector set at 280 nm. Separation was achieved on an Eclipse C18 column (4.6 mm × 250 mm, 5 µm particle size). The mobile phase consisted of water (solvent A) and 0.05% trifluoroacetic acid in acetonitrile (solvent B), delivered at a flow rate of 0.9 mL/min. The mobile phase was programmed using a segmented linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); and 15–20 min (82% A). The injection volume was 5 µL, and the column temperature was maintained at 40 °C. Identification and quantification of phenolic compounds were based on retention times and calibration curves of authentic standards. Calibration curves were constructed for each standard phenolic compound (e.g., gallic acid, rutin, quercetin) using five concentrations ranging from 10 to 200 µg/mL. The extracts under study were analyzed in triplicate, with mean values reported in the text.

Evaluation of antioxidant capacity

The antioxidant activity of cumin and musk extracts was evaluated using several methods. Ascorbic acid was used as a positive control in all assays. The DPPH radical scavenging activity (19). The reducing power was determined using the potassium permanganate ($KMnO_4$) assay (20). The hydrogen peroxide scavenging activity (21). The ABTS radical scavenging activity (22). Additionally, the DPPH method was used to evaluate the antioxidant activity of a blend of the promising extract with ascorbic acid and expressed as the percentage inhibition of DPPH radical.

Antibacterial activity by agar well diffusion method

The antibacterial activity was assessed using the agar well diffusion method. according to the National Committee for Clinical Laboratory Standards (NCCLS) protocol. Extracts were tested against a common human pathogenic microorganism, Staphylococcus aureus (a gram-positive bacterium) and Pseudomonas aeruginosa (a gram-negative bacterium). Bacteria were maintained on Mueller-Hinton agar plates and sub-cultured on their respective media, at 37°C for 24h. All the strains screened for antibacterial activity were obtained from the American Type Culture Collection (ATCC). The bacterial culture was spread on Muller-Hinton agar plates using a sterile swab moistened with a bacterial suspension. Sterilized 8 mm diameter wells were punched into the agar medium using sterilized test tubes and forceps. The wells were then filled with 100 µL of plant extract (25 mg/mL) and allowed to diffuse at room temperature for 2 hours. Four plates were prepared for each bacterium, each with a different concentration of plant extract. After incubation at 37°C for 24 hours, the inhibition zone around each well was measured. Pure media plates were used as negative controls. The values were compared with positive controls (Gentamycin (10µg/mL) and Ofloxacin (5µg/mL) synthetic bactericidal drugs) for antibacterial susceptibility. Each experiment was performed in triplicate, and the mean values of the inhibition zones were calculated with standard errors.

In vitro antiviral activity by MTT assay.

The antiviral activity was evaluated using the MTT assay on Vero E6 cells infected with human coronavirus 229E with minor modifications (23,24). The IC_{50} and CC_{50} values were calculated from dose-response curves.

Cytotoxicity on normal cells (CC_{50}): Cells were seeded in 96-well plates (100µL/well at a density of 3×10^5 cells/mL) and incubated for 24 hours at 37°C in 5% CO_2. The cells were then treated with various concentrations of the extracts (100µL) in triplicate. Twenty-four hours later, the supernatant was discarded, the cell monolayers were washed three times with sterile 1x phosphate-buffered saline (PBS), and 20µL of MTT stock solution (5mg/mL) was added to each well and incubated at 37°C for 4 hours. After incubation, the medium was aspirated, and the formazan crystals were dissolved with 200µL of acidified isopropanol (0.04 M HCl in absolute isopropanol). The absorbance of the formazan solutions was measured at a wavelength of 540 nm with a reference wavelength of 620nm using an Anthos Zenyth 200rt plate reader (Anthos Labtec, Heerhugowaard, Netherlands). The percentage of cytotoxicity, relative to untreated cells, was calculated as a percentage of viability and plotted versus concentration for each mixture using GraphPad Prism 5 software. All experiments were performed in triplicate.

Antivrial activity (IC_{50}): The IC_{50} values for both extracts were determined. Briefly, 2.4 × 104 Vero-E6 cells were seeded in each well of a 96-well tissue culture plate and incubated overnight at 37°C in a humidified 5% CO_2 incubator. The cell monolayers were washed once with 1x PBS and exposed to 229E virus (100 $TCID_{50}$) for 1 hour at room temperature. The cell monolayers were overlaid with 50µL of DMEM containing varying concentrations of the extracts. The plates were incubated at 37°C in a 5% CO_2 incubator for 72 hours. The cells were fixed with 100 µL of 4% paraformaldehyde for 20 minutes and stained with 0.1% crystal violet in distilled water for 15 minutes at room temperature. The crystal violet dye was dissolved with 100µL of absolute methanol per well and the optical density of the color was measured at 570 nm. Viral inhibition (%) versus concentration for each mixture and IC50 was determined from the obtained curve.

Fractionating bioactive ingredients of the promising extract by Column chromatography

A 40cm long, 2.5cm diameter column packed with 150g of silica gel (60-120 mesh) was used for the separation of the ethanolic extract of musk. The extract (1.2g) was ground with silica gel powder and placed on top of the column. The column was eluted sequentially with 100% hexane, ethyl acetate, and ethanol with the polarity of the mobile phase mixtures increased by 30% between each elution step. This resulted in a total of 8 fractions.

Statistical analysis

Data are expressed as the mean ± standard error (SE) for three replicates. Statistical analysis involved a one-way analysis of variance (ANOVA). A value of P less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Herbs and spices contain bioactive compounds that have potential medicinal uses. These compounds can have antioxidants, anticancer, antimicrobial, anti-inflammatory, and other health benefits (25,26).

Phytochemical Screening of Cumin and Musk Extracts

The presence of major phytochemical families was initially assessed. **Error! Reference source not found.** shows the results of the qualitative phytochemical screening of the extracts. Both extracts contain high levels of phenols and flavonoids. However, alkaloids, anthocyanins, and coumarins were not detected. Numerous studies have examined the phytochemicals in musk and cumin

extracts, identifying phenols, flavonoids, and alkaloids in *Cuminum* extract (27). While previous studies have reported the presence of alkaloids in *Cuminum* extract, our analysis did not detect these compounds. This variation may be attributed to environmental factors influencing secondary metabolite biosynthesis.

Table 1: Qualitative phytochemical screening of cumin and musk ethanolic extracts

Compound	Inference	
	E. moschata (Musk)	C. *cyminum* (cumin)
Phenols	+	+
Flavonoids	+	+
Coumarins	-	-
Anthocyanins	-	-
Alkaloids	-	-

(+) present and (–) absent.

Quantitative Analysis of Phenolic and ascorbic acid compounds

Total phenolic, flavonoid, condensed tannin, and ascorbic acid contents were quantified. Cumin showed higher levels of phenols, flavonoids, and tannins, while musk had greater ascorbic acid content. (Figure 1 and table 2). Ascorbic acid exhibits concentration-dependent antioxidant activity, with peak activity observed at 400 µg/mL. (28). Cumin seed oil was found to contain ~ 85mg gallic acid equivalents/g (GAE/g) of total phenols and ~52mg quercetin equivalents/g (QE/g) of flavonoids (8).

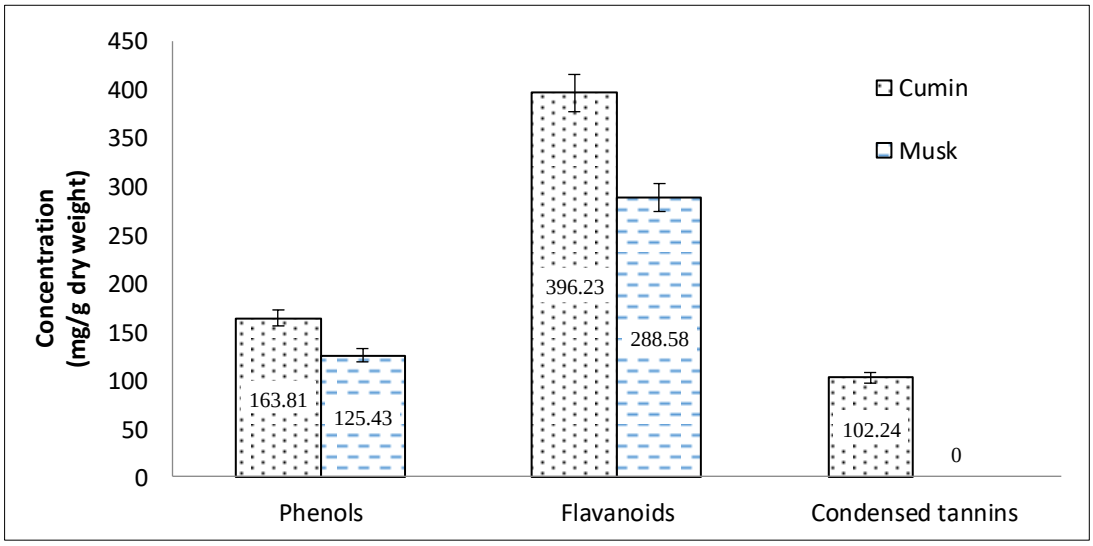


Figure 1. Phenolic, flavonoid, and condensed tannin content (mg/g dry weight) of cumin (blue color) and musk (red color) ethanolic extracts.

Table 2: Ascorbic Acid Content of Ethanol Extracts from *E. moschata* and *C. cyminum*.

Plant extract	Ascorbic acid (mg/mL)
E. moschata (Musk)	0.486 ± 0.02^{a}
C. cyminum (Cumin)	0.243 ± 0.01^{b}

Antioxidant Activity

Antioxidant activity of both extracts was examined with a variety of methods. Integrating results from multiple methods offers a more comprehensive understanding of the extract's overall antioxidant capacity. Each method evaluates distinct aspects of antioxidant activity, such as free radical scavenging, reducing power, and metal chelation. Employing various methods minimizes biases associated with individual techniques and enhances the reliability and credibility of the findings (29, 30). Therefore, several methods have been used, i.e., DPPH, ABTS, $KMnO_4$, H_2O_2, and methylene blue assays.

As **Error! Reference source not found.**3 shows, cumin ethanolic extract demonstrated stronger antioxidant activity than musk ethanolic extract in ABTS, $KMnO_4$, and methylene blue assays. Conversely, musk ethanolic extract exhibited greater antioxidant activity in DPPH and H_2O_2 assays compared to cumin ethanolic extract. These findings highlight the distinct antioxidant profiles of cumin and musk extracts. While both extracts exhibit antioxidant properties, they appear to achieve this through different mechanisms and with varying levels of specific compounds.

Spice extracts work as natural antioxidants and antimicrobials, often as good as or better than synthetic ones (31). Spices are rich in phenolic compounds with strong antioxidants and anti-inflammatory properties, offering health benefits like fighting infections, reducing oxidative stress and inflammation, and aiding in conditions such as diabetes, cancer, neurodegenerative, and cardiac disorders (32).

Table 3: Antioxidant Activity (%) of Cumin and Musk Ethanolic Extracts.

Antioxidant assay	Antioxidant activity of 100µg/mL extract (%)	
	E. moschata (Musk)	C. *cyminum* (Cumin)
DPPH	66.64 ± 3.33^{a}	26.68 ± 1.33^{b}
ABTS	62.3 ± 3.12^{b}	80.9 ± 4.05^{a}
$KMnO_4$	0.77 ± 3.90^{b}	19.08 ± 0.95^{a}
Methylene blue	55.8 ± 2.79^{b}	70.24 ± 3.51^{a}
H_2O_2	22.7 ± 1.14^{a}	17.11 ± 0.85^{b}

The ascorbic acid content in musk extract was remarkably high, containing twice as much vitamin C as cumin extract. This significant difference prompted us to further investigate its antioxidant properties.
Figure 2 demonstrates the potent antiradical activity of the musk ethanolic extract. At a concentration of 100 µg/mL, the extract rapidly scavenged the DPPH radical, reducing absorbance from 0.623 to 0.003 within 20 seconds. The extremely rapid kinetics of DPPH reduction - a 99.5% decrease within 20 seconds - suggest the presence of highly reactive antioxidant compounds. The efficiency of the ethanol solvent in extracting polar compounds contributed to the isolation of these potent bioactive agents. The rapid reaction kinetics indicate a direct and highly efficient interaction with DPPH, reflecting a high reaction-rate constant.
Although musk is less recognized as a medicinal plant, this is likely due to its widespread use as a spice and its ease of cultivation across many regions worldwide that has naturally drawn greater attention to its medicinal properties and research. However, this study suggests that musk extract may possess promising bioactive properties, warranting further investigation to evaluate its medicinal potential. Musk demonstrated higher antioxidant capacity than cumin, consistent with findings by Prajapati et al., who reported that cumin extract exhibited stronger DPPH radical scavenging activity compared to black cumin, highlighting its superior antioxidant potential (27).
Another vital medicinal property of plant phytochemicals with significant benefits for human health and the environment is their antibacterial activity. This property holds great potential for reducing antibiotic usage and combating the growing issue of antibiotic resistance. The antibacterial activity observed in our cumin extract is supported by Sharifi et al., who demonstrated that cumin essential oil exhibited potent antibacterial and antivirulence effects against multidrug-resistant *S. aureus*, including inhibition of biofilm formation and quorum sensing (28). This activity is likely attributed to the presence of phenolics and flavonoids - major constituents identified in our phytochemical screening and consistent with previous studies - known for their antimicrobial properties (29).

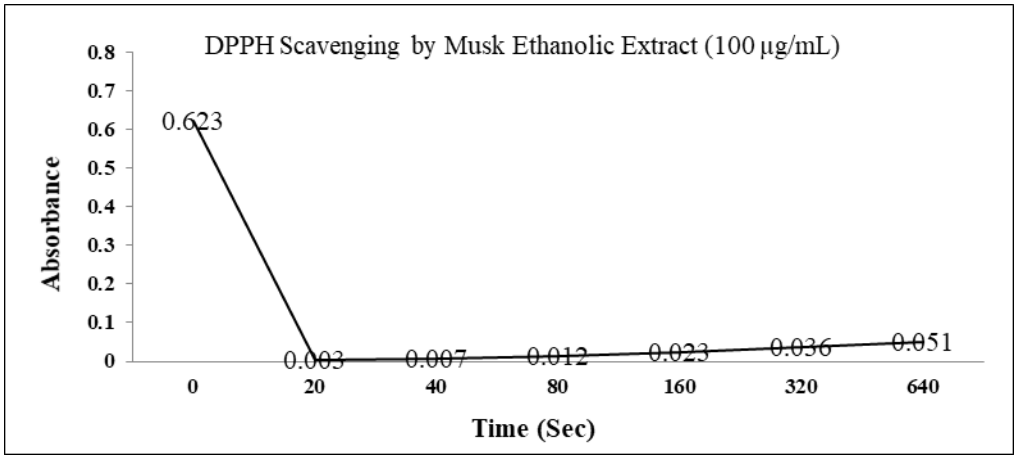


Figure 2. Antiradical activity of musk ethanolic extract at concentration of 100 µg/mL by DPPH assay.

Moreover, an experiment was conducted to compare the antioxidant activity of musk ethanolic extract, vitamin C, and a mixture of the two (**Error! Reference source not found.**4). The results further confirm that the antioxidant capacity of musk ethanolic extract is remarkably high compared to vitamin C. The antioxidant activities of pure vitamin C (72.55 ± 3.63%), the mixture of 0.2 mL extract plus 0.8 mL vitamin C (69.9 ± 3.50%), and the pure extract (63.3 ± 3.17%) were measured. Even though there is a significant difference between pure vitamin C and the extract, the extract's antioxidant capacity is still remarkably high compared to vitamin C.

Table 4: Hybrid reaction (%) of musk ethanolic extract with vitamin C against DPPH.

Samples	Antioxidant activity (%) at 100µg/mL
1 mL extract	63.3 ± 3.17^{d}
0.8 mL extract + 0.2 mL Vit. C	66.4 ± 3.32^{c}
0.6 mL extract + 0.4 mL Vit. C	68.84 ± 3.44^{b}
0.4 mL extract + 0.6 mL Vit. C	69.73 ± 3.49^{b}
0.2 mL extract+ 0.8 mL Vit. C	69.9 ± 3.50^{b}
1 mL Vit. C	72.55 ± 3.63^{a}

The values are the average of three measurements for each parameter ± SE. Different superscript letters indicate a significant difference ($P < 0.05$).

Antibacterial Activity against human pathogens

The antibacterial activity of cumin and musk ethanolic extracts was evaluated using the well-diffusion method against common human pathogens, namely *S. aureus* (Gram-positive bacteria) and P. aeruginosa (Gram-negative bacteria), by measuring the presence or absence of inhibition zone diameters.

The data in **Error! Reference source not found.** indicates that Cumin extract exhibited higher antibacterial activity against both tested bacteria compared to Musk, although it was less effective than the standard antibiotic Ofloxacin. The antibacterial efficacy of cumin extract was approximately 87% and 73% against S. aureus and P. aeruginosa, respectively. Similarly, musk extract demonstrated significant antibacterial activity, with effectiveness of 64% and 50% against S. aureus and P. aeruginosa, respectively, in comparison to the more potent antibiotic Ofloxacin.

Table 5: Antibacterial activity of cumin and musk ethanolic extracts.

Tested Bacteria	Zone of inhibition (mm) ± standard error			
	E. moschata (Musk)	*C. cyminum* (Cumin)	Standard	
			Gentamycin (10µg/mL)	Ofloxacin (5µg/mL)
1 - *Staphylococcus aureus*	14 ± 1.0 (64%)	16 ± 2.0 (73%)	19 ± 1.5	22 ± 0.0
2 - *Pseudomonas aeruginosa*	12 ± 1.5 (50%)	21 ± 2.5 (87%)	18 ± 2.0	24 ± 2.0

The values are the average of three measurements for each parameter ± SE.

Previous studies have shown that cumin exhibits significant activity against various bacteria, including *Salmonella typhi*, *Escherichia coli*, and *Enterobacter aerogenes*, with potency in some cases exceeding that of the standard antibiotic chloramphenicol (30). The cumin extract demonstrated both antibacterial and antifungal properties against various pathogenic bacteria and fungi, with the strongest antibacterial

effect observed against E. coli and P. aeruginosa (6). Another study found that cumin seed oil treatment improved the health of mice infected with toxoplasmosis, a disease caused by a parasite. The improvement was like that seen with standard antibiotic treatments (8).

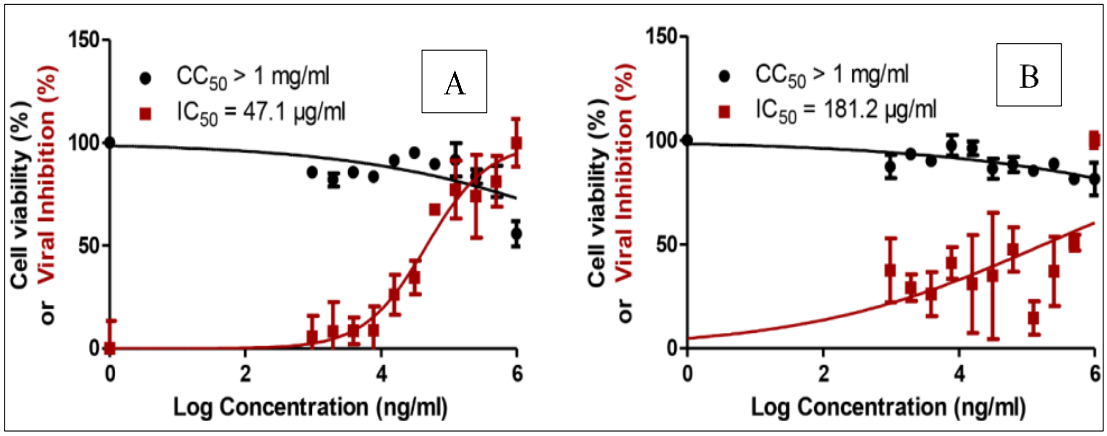


Figure 3. Antiviral activity of musk (A) and cumin (B) ethanolic extracts tested on Vero E6 cells.

Antiviral Activity against Human Coronavirus 229E

Antiviral activity represents a significant challenge, as viruses infect host cells and can escape the immune system by constantly evolving, with new strains emerging regularly. Scientists are continually striving to combat viral infections, making plant phytochemicals with antiviral properties highly valuable. The MTT assay is a common method used to measure cell viability and cytotoxicity. It is often used in drug discovery to screen for new drugs. In this study, the MTT assay was used to assess the antiviral activity of cumin and musk ethanolic extracts against the 229E virus using Vero E6 cells. Figure 3 shows that musk ethanolic extract exhibited moderate antiviral activity, while cumin showed low antiviral activity. Both extracts were highly safe for normal cells, as their CC_{50} values were higher than 1 mg/mL. However, the IC_{50} values for viral inhibition were remarkably high for both extracts, at 47µg/mL for musk and 181 µg/mL for cumin, respectively. The antiviral activity of musk was about 385 times that of cumin extract, based on the IC_{50} values, indicating that musk extract is a highly promising candidate as potential antiviral drug development.

Fractionation and Antioxidant Activity of Musk Extract

Due to the promising antiviral, antioxidant, and antibacterial properties exhibited by musk extract, we chose to undertake a more in-depth analysis of its chemical composition. The extract was fractionated into eight fractions using column chromatography with different solvent systems. The antioxidant activity of these fractions was then evaluated using ABTS and methylene blue assays. As shown in Table 6, fraction 8 (100% ethanol) exhibited the highest antioxidant activity, with 99.6 ± 4.98% inhibition of ABTS radicals and 100% complete reduction. It also demonstrated the highest antioxidant activity in the methylene blue assay, with a value of 61.003 ± 3.05. This may be due to the radical scavenging compounds that are often found in water and polar solvent fractions (31). On contrast, the chloroform extract of cumin showed excellent antioxidant activity (6).

Table 6: Antioxidant activity (%) of E. moschata ethanolic extract fractions.

Fraction No.	Solvent systems	% Antioxidant activity			
		ABTS		Methylene blue	
		1 min	10 min	15 min	30 min
1	100% Hexane	5.4 ± 0.27	80.3 ± 4.02	21.29 ± 1.06	5.60 ± 0.28
2	70% Hex: 30% EA	22.1 ± 1.1	1.19 ± 0.06	27.27 ± 1.36	22.42 ± 1.12
3	40% Hex: 60% EA	0.94 ± 0.05	24.9 ± 1.25	37.92 ± 1.90	33.43 ± 1.67
4	10% Hex: 90% EA	7.6 ± 0.38	4.85 ± 0.24	12.31 ± 0.62	32.87 ± 1.64
5	100% Ethyl Acetate	7.9 ± 0.40	5.02 ± 0.25	27.70 ± 1.39	37.23 ± 1.68
6	70% EA:30% ethanol	5.0 ± 0.1	5.0 ± 0.1	22.85 ± 1.14	35.80 ± 1.79
7	40% EA:60% Ethanol	45.78 ± 2.29	62.53 ± 3.13	79.6 ± 3.98	55.92 ± 2.80
8	100% Ethanol	99.6 ± 4.98	99.5 ± 4.98	87.52 ± 4.38	61.003 ± 3.05

HPLC Profiling of Phenolic Constituents

The phenolic compounds in cumin and musk ethanolic extracts were identified and quantified using HPLC (**Error! Reference source not found.**7, Figures 4-5). The results show that cumin and musk extracts have different phenolic profiles, but both contain compounds that are known for their antioxidant properties and potential health benefits.

For example, Cumin extract contained rutin, methyl gallate, chlorogenic acid, and vanillin as major compounds, while musk extract contained ellagic acid as the major compound. Other phenolic compounds, such as catechin, methyl gallate, syringic acid, ferulic acid, daidzein, quercetin, and apigenin, were found only in cumin extract. Cinnamic acid was found only in musk extract.

The Cumin HPLC peak shows a compound corresponding to the peaks at 10.711 min and 11.349 min need to be identified. This could be done by using a mass spectrometer (HPLC-MS) coupled with the HPLC system. The presence of vanillin, although in a relatively small amount, is also notable. The area under the curve (AUC) for Chlorogenic acid, Querectin and Apigenin are provided (705.589, 510.856 and 723.814 respectively) and allow the relative quantification of those two compounds. Previous studies have shown that cumin seeds contain phenols, such as salicylic, cinnamic, gallic, p-hydroxybenzoic acid, hydroquinone, and resorcinol, as well as flavonoids, such as rutin, quercetin, and coumarin (33,34).

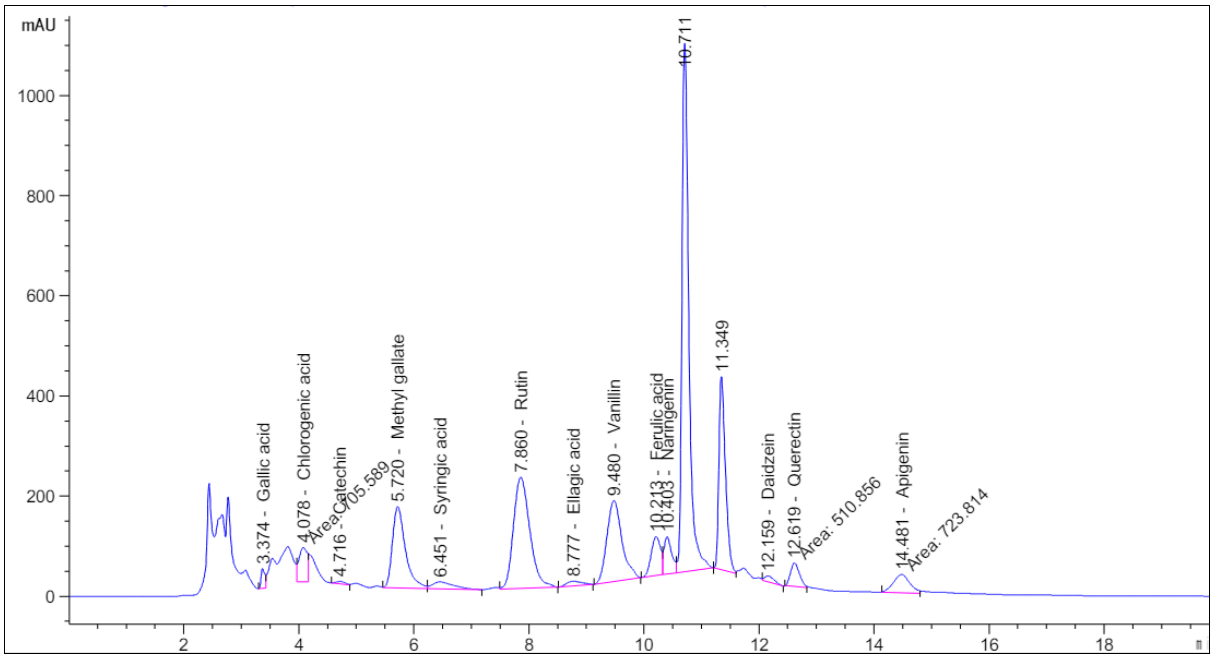


Figure 4.HPLC chromatogram of Cumin ethanolic extract.

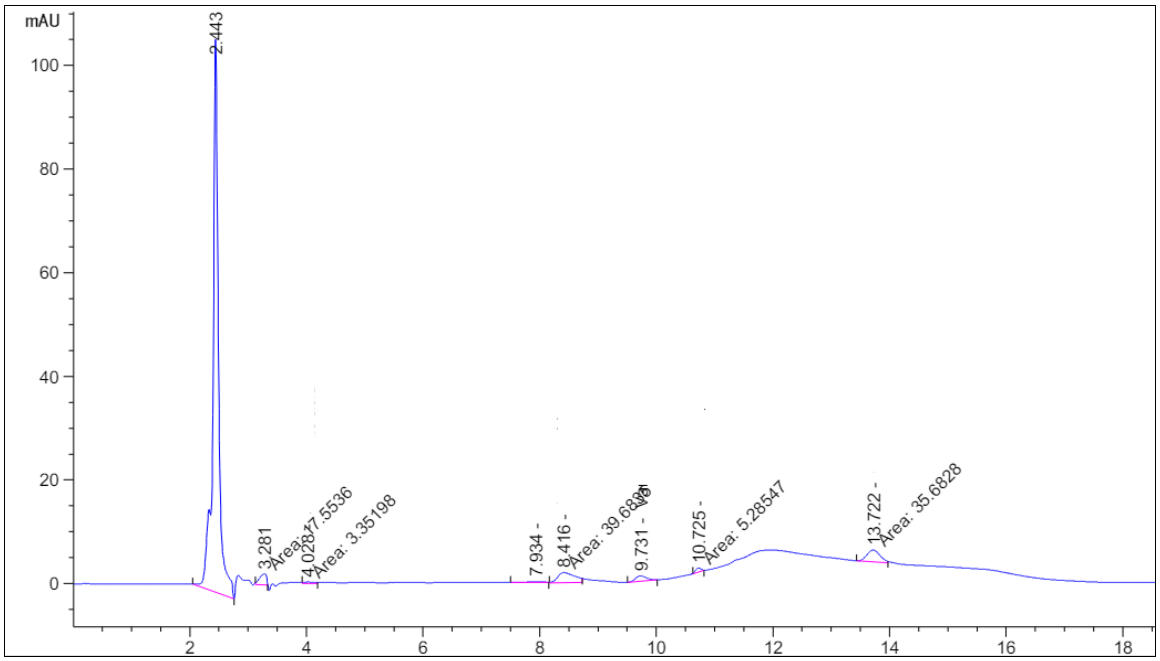
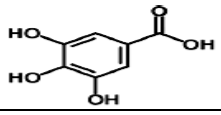
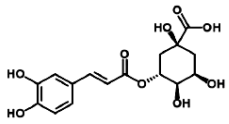
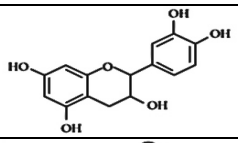
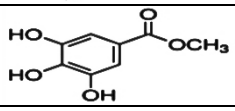
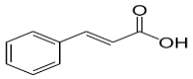
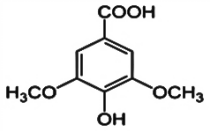
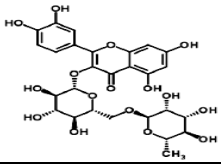
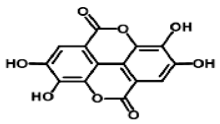
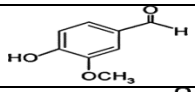
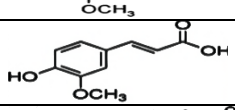
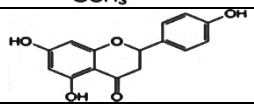
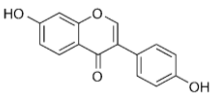
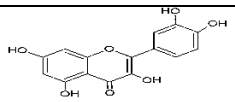
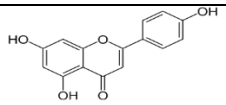


Figure 5. HPLC chromatogram of Musk ethanolic extract.

Table 7: HPLC analysis of phenolic compounds in cumin and musk ethanolic extracts (µg/ml).

Phenolic compound	Chemical structure	Concentration (µg/ml)	
		E. moschata **(Musk)**	**C. *cyminum* (Cumin)**
Gallic acid		1.32	13.66
Chlorogenic acid		0.48	100.35
Catechin		ND	12.84
Methyl gallate		ND	143.92
Cinnamic acid		0.50	ND
Syringic acid		ND	26.62
Rutin		0.40	518.43
Ellagic acid		10.35	44.58
Vanillin		0.40	88.91
Ferulic acid		ND	45.63
Naringenin		0.50	66.71
Daidzein		ND	8.30

Phenolic compound	Chemical structure	Concentration (µg/ml)	
Querectin		ND	61.84
Apigenin		ND	42.14

Compared to the cumin extract, the musk extract shows significantly fewer distinct peaks. This suggests a less complex mixture of compounds. The peak areas are provided for several peaks, but without knowing the identity of the compounds, it's difficult to draw specific conclusions beyond their relative abundance. The peak at 2.443 min is significantly higher than any other peak. This suggests that the musk extract is dominated by a single compound that elutes very early in the chromatographic run. Identifying the compound corresponding to this peak by HPLC-MS is crucial to determine its molecular weight and fragmentation pattern for accurate identification.

Natural antioxidants from plant phytochemicals help prevent diseases like cancer, cardiovascular and cataracts, while boosting the immune system and protecting against damage from free radicals. Growing awareness highlights the role of antioxidants in boosting the body's defenses, preventing oxidative stress-related diseases, and enhancing anticancer treatments. (28).

Conclusion

This study investigated the phytochemical composition and biological activities of cumin and musk ethanolic extracts. Both extracts were found to contain a variety of bioactive compounds, particularly phenols and flavonoids, and showed strong antioxidant activity. The antibacterial activity of cumin and musk ethanolic extracts was evaluated using the well-diffusion method against common human pathogens. Cumin extract exhibited stronger antibacterial activity against both S. aureus and P. aeruginosa compared to musk extract, although both were less effective than the standard antibiotic Ofloxacin. The antiviral activity of musk extract against the human coronavirus 229E was significantly higher than that of cumin extract. Both extracts showed low cytotoxicity toward normal cells. Further fractionation of musk extract revealed that the fraction obtained with 100% ethanol exhibited the highest antioxidant activity, indicating the presence of potent radical scavenging compounds in this fraction. These findings highlight the potential of cumin and musk extracts, particularly musk, as natural sources of bioactive compounds. However, further in vivo and clinical studies are necessary to validate their therapeutic applications. While the in vitro results are encouraging, the findings should be considered preliminary. Comprehensive in vivo studies and clinical trials are essential to confirm the safety, efficacy, and therapeutic relevance of these extracts.

Availability of data and materials:

They are available as Supporting information.

Ethics approval and consent to participate:

Not applicable in this section.

Consent for publication:

All authors read and approved the final manuscript.

Conflicts of Interest:

The authors declare no conflict of interest.

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