*Egypt. J. Chem.* **Vol. 68**, No. 5 pp. 149 - 165 (2025)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Evaluation of Antiviral and Antimicrobial Properties of Chenopodium murale Extracts: A Potential Natural Source for Health and Medical Applications

**Gihan M. Zaghlol[*1], Mohsen A. Sayed[2], Sayed M. S. Abo El-Souad[2], Amena S. El-Feky[3], Sohaila S. Awad[4], Fatma R. Elfiky[3], Maram A. Abdelghany[5], Hanaa H.B. Elmalla[6], Heba M. Fahmy[3*]**

[1] *Department of Botany and Microbiology, Faculty of Science, Helwan University, Ain Helwan, Cairo, Egypt*
[2] *Botany and Microbiology Department, Faculty of Science, Cairo University, Cairo, Egypt*
[3] *Biophysics Department, Faculty of Science, Cairo University, Cairo, Egypt*
[4] *Chemistry and Biochemistry Department, Faculty of Science, Cairo University, Cairo, Egypt*
[5] *Biotechnology Department, Faculty of Science, Cairo University, Cairo, Egypt*
[6] *Chemistry and Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt*

Abstract

**Background:** Chenopodium murale (C. murale), a plant used in herbal medicine, is rich in essential oils and bioactive compounds. Given the urgent need for natural sanitisers and disinfectants, this study aimed to evaluate the antiviral and antimicrobial properties of various C. murale extracts and to develop an adequate, low-toxicity, ready-to-use green preparation.

**Methods:** Crude extracts of C. murale prepared with ethanol, methanol, acetone, ethyl acetate, and hexane were tested for cytotoxicity, antiviral, and antimicrobial activities. Liquid Chromatography/Mass Spectrometry was used to identify the metabolites in the most potent extract.

**Results:** The ethanol extract of C. murale showed promising antiviral activity, particularly against adenovirus type 7, without significant toxicity to tested cell lines. Identified compounds in the crude ethanolic extract included pentoses, hexoses, amino acids, flavonoids, carotenoids, terpenoids, sterols, and glycosides. The essential oil derived from C. murale, containing p-cymene and a-terpinene, demonstrated antimicrobial properties. Combined with other essential oils, the plant extracts showed activity against various microbial species, aligning with previous research on the antimicrobial effects of C. murale.

**Conclusion:** This study highlights the potential of C. murale and its ethanolic extract as a potent natural antiviral source for various health and medical applications.

Keywords: Antimicrobial; Antiviral; Chenopodium murale; Coronavirus; Cytotoxicity; Metabolite Profiling.

## I. Introduction

In the fields of health and medicine, the quest for potent antiviral and antibacterial agents is of paramount importance. Recently, there has been a surge in interest in exploring natural sources for potential medicinal applications. Chenopodium murale (C. murale), a plant traditionally used in herbal medicine, has emerged as a promising candidate due to its reported therapeutic benefits [1].

C. murale is renowned for its rich concentration of bioactive compounds and essential oils, including minerals, albuminoids, phenolic amide, saponins, sitosterol (found in the roots), and oleanolic acid (found in the flowers) [2-4]. These substances have been associated with various biological activities and medicinal properties. Given the urgent need for natural sanitisers and disinfectants, it is logical to explore the antiviral and antibacterial potentials of C. murale extracts [5].

In the present study, we employed various extraction methods using solvents such as ethanol, methanol, acetone, ethyl acetate, and hexane to evaluate the potential of C. murale. The resulting crude extracts were tested extensively to assess their antibacterial, antiviral, and cytotoxic properties. Liquid Chromatography/Mass Spectrometry analysis was used to identify and characterize the metabolites in the extracts, providing insights into their chemical composition and potential therapeutic benefits.

In our study, the extracts were tested on various cell lines, including human larynx epidermoid carcinoma cells and Buffalo green monkey kidney cells, to determine their cytotoxicity. The antibacterial activity was evaluated individually and with other enhancing agents such as aloe vera and essential oils. The antiviral activity was assessed against bacteriophage phiX174 virus, adenovirus type 7, and coxsackievirus B4. This study highlights the potential of C. murale extracts as a valuable natural resource for health and medicinal applications [5].

*Corresponding author e-mail: hfahmy@sci.cu.edu.eg (Heba M. Fahmy).
Received date 15 June 2024; revised date 09 September 2024; accepted date 12 September 2024
DOI: 10.21608/ejchem.2024.293838.9789

Chenopodium murale extracts show unique beneficial attributes with some similarities, especially in relation to other plant extracts such as Rindera lanata and Bidens pilosa due to their broad-spectrum chemical composition, low cytotoxicity, and antiviral and antibacterial properties [6-7].
The abundance of bioactive compounds in C. murale and the demonstrated antiviral activity make it a promising candidate for developing potent and sustainable medicinal agents. This research contributes to the growing body of knowledge on natural product-based drug development by investigating the antiviral and antibacterial capabilities of C. murale extracts, emphasizing the importance of harnessing nature's resources to improve human health.

## 2- Material and Methods

### 2.1 Materials

After obtaining the required owner permits, a new sample of *Chenopodium murale* was gathered in February 2021 from growing areas in Egypt. Sigma Aldrich provided the study's chemicals: ethanol 99.9%, methanol 99.9%, acetone 99.9%, hexane, and ethyl acetate. Furthermore, local vendors provided the essential oils, which included Aloe vera, Cinnamon, Clove, Lavender, Lemon, Neem, Peppermint, and Green Tea. Notably, this study complies with all applicable municipal and federal laws on the use of plants.

### 2.2 Methods

#### 2.2.1 Collection and Identification of Chenopodium Murale Plant

The leaves, stems, and flowers of *Chenopodium murale* were gathered, divided, cleaned, and thoroughly washed with distilled water. Considering the hot and dry weather, They were left to air dry for around three days. The plant material was finely powdered and ready for extraction after drying [8]. The specimen has been identified, and the number cai was given. 24.71.128.381. The senior taxonomist verified and certified that the plant was, in fact, *Chenopodiastrum murale (L.) S. Fuentes*, Uotila & Borsch. According to Plants of the World online, the plant was registered under the new name *Chenopodiastrum murale (L.) S.Fuentes*, Uotila & Borsch and is kept in the Cairo University Herbarium.

#### 2.2.2 Preparation of Crude Extracts

The plant powder was combined with ethanol, methanol, and acetone at 1:10 w/v [9, 10] and hexane and ethyl acetate at 1:5 w/v [11, 12]. The extracts were then macerated in the appropriate solvents and shaken for a whole day at 150 rpm [13].
After the extraction procedure, the extracts were lyophilized and filtered through Whatman® grade 2 filters [13]. To ensure the extracts were completely dry and the weights were constant, they were left to sit for 48 hours. The extracts were placed into sterile glass containers and refrigerated until further use to facilitate future research.

#### 2.2.3 Liquid Chromatography/ Mass Spectrometry (LC-ESI-MS/MS) Analysis

Five crude extracts from the *C. murale* sample were generated to cover the wide range of polarity exhibited by plant metabolites and ensure thorough coverage for the study objectives. The SCIEX® Triple Quad 5500+ MS/MS system, which included an electrospray ionization (ESI) detector for "soft ionization" [ESI LC-MS/MS], was used to analyze the samples. An ExionLC AC system and an Ascentis® C18 Column (4.6 x 150 mm, 3 µm) were used to achieve separation.
Two eluents made up the mobile phase were A, acetonitrile (LC grade), and B, 5 mM ammonium formate in 0.1% formic acid (pH 8 adjusted with NaOH for negative mode). The mobile phase gradient was programmed as follows: 10% B for the first minute, increasing from 10% to 90% B over the next 33 minutes, maintaining 90% B for an additional 37 minutes, then returning to 10% B at 37.1 minutes, and holding at 10% B for the remaining 40 minutes. The injection volume was set at 10 µL, with a flow rate of 0.7 ml/min.
Both positive (PI) and negative (NI) ionization modes were used for the MS/MS analysis, with an EMS-IDA-EPI scan from 100 to 1000 Da for MS1. The parameters were as follows: ion source gas 1 and 2 set at 45 pressure, curtain gas at 25 psi, source temperature for both modes at 500°C, and IonSpray voltage at 5500 for positive mode and -4500 for negative mode. The specifications for MS2 comprised a declustering potential of 80 and -80, collision energy of 35 and -35, and collision energy spread of 20 and 15 for PI and NI, respectively. The scan range was 50 to 800 Da.
Version 4.70 of the MS-DIAL program was used to identify the chemicals, enabling precise identification and analysis of the data.

#### 2.2.4 Cytotoxicity test

##### 2.2.4.1 Cell morphology evaluation by inverted light microscopy:

Following the procedures outlined by Simoes et al. (1999) and Walum et al. (1990) [14, 15], the experimental process was carried out. Hep-2 and BGM cell cultures were established separately on 96-well tissue culture plates (Greiner-Bio One, Germany) with a $2\times10^5$ cells/mL density. After that, the plates were incubated for a full day at 37°C with 5% (v/v) $CO_2$ in a humidified environment. The media in each well was removed once the cell monolayers had reached confluency throughout this incubation period. The tested Sample was prepared in DMEM (GIBCO BRL), and 100 µL of bi-fold dilutions were added to each well. 100 µL of DMEM without any samples was added for the cell controls. Then, all cultures were further incubated for 72 hours at 37°C in a humidified environment with 5% (v/v) $CO_2$.
Every day during incubation, the cell morphology was examined under a microscope for any noticeable morphological changes. These changes included cytoplasm granulation and vacuolization, loss of confluence, and rounding and shrinkage of the cell. The morphological alterations that were seen were noted and given a score correspondingly [16].

The trypan blue dye exclusion technique [17] was used to evaluate the vitality of the cells. In 12-well tissue culture plates (Greiner-Bio One, Germany), Hep-2 and BGM cell cultures were grown at $2\times10^5$ cells/mL density. After a 24-hour incubation period, the examined sample was subjected to the previously established experiment. To do this, each well was filled with 100 µL of bi-fold dilutions of the tested material. The cells were trypsinized, and the culture media was withdrawn after 72 hours of incubation. The cell suspension was then supplemented with an aqueous solution of trypan blue dye at a concentration of 0.4% (w/v). The vitality of the cells was then assessed by counting them under a phase contrast microscope.

**2.2.5 Antiviral activity**

**2.2.5.1 Quantification of infectious bacteriophage phiX174 virus**

It was performed according to" Standard Methods for the Examination of Water and Wastewater ", these methods consist of standardized protocols for collecting, conserving, and analyzing water and wastewater samples, ensuring uniformity and reliability across laboratories throughout the world by assessing dissolved solids, chlorine levels, metals, and microbial content, including total and faecal coliforms [16].

**2.2.5.2 Determination of adenovirus type 7 and Coxsackievirus B4:**

Titers using plaque assay nontoxic dilutions were mixed (100 µL) with 100 µL of different doses of adenovirus type 7 and Coxsackievirus B4 ($1x10^5$, $1x10^6$, $1x10^7$) PFU/mL. The mixture was incubated for 30 min at 37 ºC. The inoculation of (100 µL) 10-fold dilutions of treated and untreated Adenovirus type 7 and Coxsackievirus B4 was carried out separately into Hep 2 and BGM cell lines in 12 multi-well plates after 1 h of incubation for adsorption at 37 $^oC$ in a 5% $CO_2$water vapour atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 mL of 2X media (Dulbecco's Modified Eagle Medium, Gibco- BRL (DMEM) plus 1 mL 1% agarose was added to each well, and the plates were incubated at 37 $^oC$ in a 5% $CO_2$ vapour atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formalin fixation, and the number of plaques was counted. The viral titers were calculated and expressed as PFU/mL 1171. The environmental condition during the Test Methods was 24 $^oC$.

**2.2.6. Antimicrobial activity**

**2.2.6.1 Test organisms**

Five bacterial species were used in this study, which were: *Pseudomonas aeruginosa* (ATCC10145), *Enterococcus faecalis* (ATCC19433), *Mycobacterium smegmatis* (ATCC19420), *Bacillus cereus* (ATCC1080), and *Staphylococcus aureus* (ATCC6538). Additionally, six fungal species, *Aspergillus flavus* (ATCC9643), *A. niger* (ATCC1015), *A. sydowii* (ATCC16844), *A.ustus* (ATCC1041), *Candida albicans* (EMCC105), and *Saccharomyces cerevisiae* (ATCC9763) were tested as well.

**2.2.6.2 Assay of antimicrobial activity of *C. murale* extracts**

The Agar disc diffusion method was used. Paper discs were impregnated with 5uL of *C. murale* crude extract of 200 µg from each extract dissolved in 1mL of diluted DMSO (0.1%), and each disc was loaded with it. The discs were placed on Mueller-Hinton agar plates and Nutrient agar inoculated with fungal and bacterial species, respectively. Plates were incubated at 37 $^oC$ for 24 hr for bacterial species and 28 $^oC$ for four days for fungal species. The inhibition zone diameters (mm) were measured. Ciprofloxacin was used as a control in the case of bacteria. Tioconazole was used as a control for fungi (CLSI 2012).

**2.2.4 Cytotoxicity test**

**2.2.4.1 Cell morphology evaluation by inverted light microscopy:**

Following the procedures outlined by Simo•es et al. (1999) and Walum et al. (1990) [14, 15], the experimental process was carried out. Hep-2 and BGM cell cultures were established separately on 96-well tissue culture plates (Greiner-Bio One, Germany) with a $2\times10^5$ cells/mL density. After that, the plates were incubated for a full day at 37°C with 5% (v/v) $CO_2$ in a humidified environment. The media in each well was removed once the cell monolayers had reached confluency throughout this incubation period. The tested Sample was prepared in DMEM (GIBCO BRL), and 100 µL of bi-fold dilutions were added to each well. 100 µL of DMEM without any samples was added for the cell controls. Then, all cultures were further incubated for 72 hours at 37°C in a humidified environment with 5% (v/v) $CO_2$.

Every day during incubation, the cell morphology was examined under a microscope for any noticeable morphological changes. These changes included cytoplasm granulation and vacuolization, loss of confluence, and rounding and shrinkage of the cell. The morphological alterations that were seen were noted and given a score correspondingly [16].

**2.2.4.2 Cell viability assay**

The trypan blue dye exclusion technique [17] was used to evaluate the vitality of the cells. In 12-well tissue culture plates (Greiner-Bio One, Germany), Hep-2 and BGM cell cultures were grown at $2\times10^5$ cells/mL density. After a 24-hour incubation period, the examined sample was subjected to the previously established experiment. To do this, each well was filled with 100 µL of bi-fold dilutions of the tested material. The cells were trypsinized, and the culture media was withdrawn after 72 hours of incubation. The cell suspension was then supplemented with an aqueous solution of trypan blue dye at a concentration of 0.4% (w/v). The vitality of the cells was then assessed by counting them under a phase contrast microscope.

**2.2.5 Antiviral activity**

**2.2.5.1 Quantification of infectious bacteriophage phiX174 virus**

It was performed according to" Standard Methods for the Examination of Water and Wastewater ", these methods consist of standardized protocols for collecting, conserving, and analyzing water and wastewater samples, ensuring uniformity and reliability across laboratories throughout the world by assessing dissolved solids, chlorine levels, metals, and microbial content, including total and faecal coliforms [16].

**2.2.5.2 Determination of adenovirus type 7 and Coxsackievirus B4:**

Titers using plaque assay nontoxic dilutions were mixed (100 µL) with 100 µL of different doses of adenovirus type 7 and Coxsackievirus B4 ($1x10^5$, $1x10^6$, $1x10^7$) PFU/mL. The mixture was incubated for 30 min at 37 $^oC$. The inoculation of (100 µL) 10-fold dilutions of treated and untreated Adenovirus type 7 and Coxsackievirus B4 was carried out separately into Hep 2 and BGM cell lines in 12 multi-well plates after 1 h of incubation for adsorption at 37 $^oC$ in a 5% $CO_2$water vapour atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 mL of 2X media (Dulbecco's Modified Eagle Medium, Gibco- BRL (DMEM) plus 1 mL 1% agarose was added to each well, and the plates were incubated at 37 $^oC$ in a 5% $CO_2$ vapour atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formalin fixation, and the number of plaques was counted. The viral titers were calculated and expressed as PFU/mL 1171. The environmental condition during the Test Methods was 24 $^oC$.

**2.2.6. Antimicrobial activity**

**2.2.6.1 Test organisms**

Five bacterial species were used in this study, which were: *Pseudomonas aeruginosa* (ATCC10145), *Enterococcus faecalis* (ATCC19433), *Mycobacterium smegmatis* (ATCC19420), *Bacillus cereus* (ATCC1080), and *Staphylococcus aureus* (ATCC6538). Additionally, six fungal species, *Aspergillus flavus* (ATCC9643), *A. niger* (ATCC1015), *A. sydowii* (ATCC16844), *A.ustus* (ATCC1041), *Candida albicans* (EMCC105), and *Saccharomyces cerevisiae* (ATCC9763) were tested as well.

**2.2.6.2 Assay of antimicrobial activity of *C. murale* extracts**

The Agar disc diffusion method was used. Paper discs were impregnated with 5uL of *C. murale* crude extract of 200 µg from each extract dissolved in 1mL of diluted DMSO (0.1%), and each disc was loaded with it. The discs were placed on Mueller-Hinton agar plates and Nutrient agar inoculated with fungal and bacterial species, respectively. Plates were incubated at 37 $^oC$ for 24 hr for bacterial species and 28 $^oC$ for four days for fungal species. The inhibition zone diameters (mm) were measured. Ciprofloxacin was used as a control in the case of bacteria. Tioconazole was used as a control for fungi (CLSI 2012).

**2.2.6.3 Assay of antimicrobial activity of plant extracts mixed with essential oils**

The Agar disc diffusion method was also used. Still, paper discs were impregnated with 2.5 µL of 200 µg of the plant extracts mixed with 2.5 µL of the following additives: Aloe Vera, Cinnamon, Clove, Lavender, Lemon, Neem, Peppermint, and Green Tea. The discs were placed on Mueller-Hinton agar plates, inoculated with bacterial and fungal isolates, and incubated under the same conditions as previously mentioned. The inhibition zone diameters (mm) were measured to detect the antimicrobial activity of the tested extracts.

**2.2.6.4 Determination of the minimum inhibitory concentration**

The serial dilution method was used to estimate the minimum inhibitory concentration of different treatments (MIC) (CLSI 2018). The studied concentrations were 25, 50, 100, and 200 µg/mL. Mueller–Hinton and Sabouraud's broth media were used for bacteria and fungi, respectively. With the help of 0.5 barium chloride McFarland turbidity standard, the test inoculum was $5\times10^4$ CFU/mL for bacteria and $10^3$ CFU spores/mL for fungi. The MIC is defined as the lowest concentration showing no growth.

## 3. Results

**3.1 Liquid chromatography/ mass spectrometry (LC-ESI-MS/MS) -based metabolite identification**

SCIEX®Triple Quad™ 5500+ System [QTRAP®] with Linear Ion Trap (**LIT**) technology incorporates Enhanced Mass Spectrometry scans (EMS) and Enhanced Product Ion scans (EPI) for compounds' screening, confirmation, and identification covering positive (PI) and negative (NI) ionization modes with high sensitivity and the best quality of data in investigating crude plant extracts. Combining both ionization modes (PI & NI) offers complementary structural information for compound characterization.

Metabolite identification using untargeted analysis is mainly achieved through mass-based search followed by manual verification. Values of m/z for molecular/Product ions were searched against the database(s). The EPI and EMS modes were viewed and checked for their presence by PeakView 1.2 and used to identify the detected metabolite. EPI spectra are highly selective and contain the complete molecular fingerprint of the analyte for identification that can be searched against existing mass spectral libraries and accessible databases such as MassBank, PubChem, ChemSpider, The Natural Products Atlas, etc.

Crude extracts were subjected to further investigations targeting the aim of the present study as cytotoxicity and antiviral capability. A decision was made to focus metabolite profiling and identification on only one extract with the best results in the previously mentioned ones, as it was a big challenge and a tedious operation to go with five crude extract analysis data in both PI and NI modes. Analysis data for the five extracts with the PI and NI ionization mode were better collectively represented by heatmaps in one Figure (Figure 1). Comparison between different solvent types and between the two ionizations (PI and NI) modes for the same solvent type showed that ethanol (EtOH) and methanol (MeOH) extracts tend to have nearly similar bands within a specific retention time (RT) regions but with different Total Ion Current (TIC) intensities. In contrast, acetone and hexane showed high similarity in band RT and TIC intensities.

This can be traced peak by beak from TIC chromatograms from a to e in Figure 2, using Enhanced Product Ion (EPI) scans. The acetone and hexane patterns were near the ethanol and methanol patterns. Ethyl acetate was far from the two patterns, with very light early to mid RTs and thick and rich late RTs bands.

Surprisingly, EtOH crude extract showed a better sensitivity towards the metabolite content over the complete scan, among the others, than MeOH crude extract, which was previously reported to be more familiar with plant extractions [18 – 21]. Enhanced Product Ion (EPI) of EtOH crude extract (Figure 2 a: Ethanol and Supplementary data list) showed high intensities of TIC peaks firstly, at earlier RTs in min. These are putatively corresponding to Quinic acid, Jasmonic acid, N-Acetylglycine, and 5-Hydroxytryptophan at 1.109, 1.659, 2.068, and 2.939 respectively, in the NI, and 1-Methyluric acid, Choline [M]+, Biuret, and N-Methylaniline, Pyrrole-2-carboxylic acid, Uracil, Phosphocholine, 1-Acetylimidazol, Ketoisovaleric acid [-3-Methyl-2-oxobutanoic acid], 2-Aminophenol, 4-Methyl-5-thiazoleethanol Guanidinoacetic acid at 1.63, 1.832, 1.998, 2.058, and 2.1048 respectively in the PI. Secondly, Retinoic acid *, Salicin*, Kaempferol*, Glycitein*, Octadecanedioic acid* [*: compounds are present in both PI and NI modes and represented by the highest TIC within the PI and/or NI mode], Bestatin, 2'-Deoxyuridine 5'-monophosphate, Docosatetraenoic acid, Palmitoylcarnitine cation, Guanosine 5'-monophosphate, Coniferylaldehyd, Luteolin, Palmitic acid, Methyl Heptadecanoic acid at 9.495, 9.596, 9.647, 10.620, 10.770, 11.421, 11.910, 11.942, 12.669, respectively were high TIC intensities in the NI which were more affluent and more varied in metabolite content than the PI. Although moderate to low TIC intensities metabolites such as Phloretin, N-Methylserine, Valine, 2and4-Hydroxyquinoline, 2-alpha-Mannobiose,1-Methylpseudouridine, Dihydrocapsaicin, Geranic acid, N-Acetylcytidine, 2-Deoxyribose, Coniferylaldehyd, Ornithine, 6,7-Dihydroxycoumarin, 3-Octanol, Cotinine, 2-Palmitoylglycerol, Isoxanthopterin, recorded the highest intensities among the others at RTs 3.424, 3.707, 5.342, 6.207, 8.272, 10.638, 11.884, 11.942, 13.237, 18.533, 19.06 min. Respectively (Figure 2 a: Ethanol and Supplementary data) in the PI. Cladribine, CGS 15943, SB 205607 (Phenylquinolines), Nialamide, Arachidic acid, and Fructose-1,6-bisphosphate were present with the highest TIC intensities at 15.691, 17.358, 19.787, 21.233 min RTs in the NI. Moreover, Lomifylline, 5-hydroxymethylcytidine, Tripelennamine, Cocaethylene, Tremulacin, Phytosphingosine, Amlexanox acid, 1,7-dimethylxanthine, alpha-Keto-gamma-(methylthio)butyric acid, Heneicosanoic acid, 2'-Deoxyguanosine, Guanosine* (in both NI and PI) and Asp-Phe were present with the highest TIC intensities at 20.185, 20.333, 20.553, 21.316, 22.506, 22.858, 24.216, 24, 445 and 24.868 min RTs respectively, in PI.

High TIC intensities were represented by dark lines on heatmaps and many peaks in the chromatogram. a: Ethanol is in Figure 2, supposing a collection of related metabolites is located in this final separation stage. For PI, Picrotin * (in both NI and PI), Limonene, Ipriflavone, Coniferylaldehyde, trans-Piceid, Brucine, Nornicotine, Thymol, 2'-O-Methylinosine, S-Adenosyl-methionine, 2,3-Dideoxyuridine, Perospirone, Luteolin-6-C-glucoside, 1-Monostearin, Ramipril, Palmitoylcarnitine cation, Nefazodone, CHEMBL1616521, Riboflavin, Geniposide, Nobiletin, Xanthinol, Salmeterol, Tremulacin, 5-Cholesten-3-beta-7-alpha-diol, Tremulacin, -(1-Pyrazolyl)-alanine, and 2,3-Dideoxyuridine Nicotinic acid* showed the highest TIC intensities and clear peaks at 27.335, 27.586, 29.031, 29.367, 31.551, 31.603, 32.973, 33.031, 34.844, 37.007, 37.064, 38.158, 39.256, 39.546, 39.656, and 39.715 min one-to-one RT in the PI. Likewise, the NI mode showed a region with many characteristic metabolites but with moderate to low intensities as follows: Cordycepin: Genistein, Glucobrassicin, 2'-Deoxyguanosine, 5'-diphosphate, Arachidonyl, Naringin, gamma-Glutamylmethionine, Luteolin-6-C-glucoside, Cytarabine, Linoleic acid, 1-Methyladenosine, 5-Methoxypsoralen, 3',5'-Cyclic dAMP/cyclic-di-GMP, Vitexin. , Lauric acid, Homoserine, Ethyl myristate, Canavanine*, Anserine at 27.072, 27.312, 31.768, 31.877, 32.1608, 34.84487, 35.56172, 35.94718, 35.94718, 36.750, 36.979, 38.137, 39.047, 39.642, 39.701, 39.761 min. RTs.

Certain dark lines from the heatmaps corresponding to high-length peaks from the chromatograms with the highest TIC intensities were only noticed in the EtOH PI. NI mode was susceptible to all extracts and rich in TIC intensities and separations, and significant similarity was seen in acetone and hexane, especially in the last region of RT, which might have occurred due to the high non-polarity nature and the high molecular weights of metabolites being separated at that time.

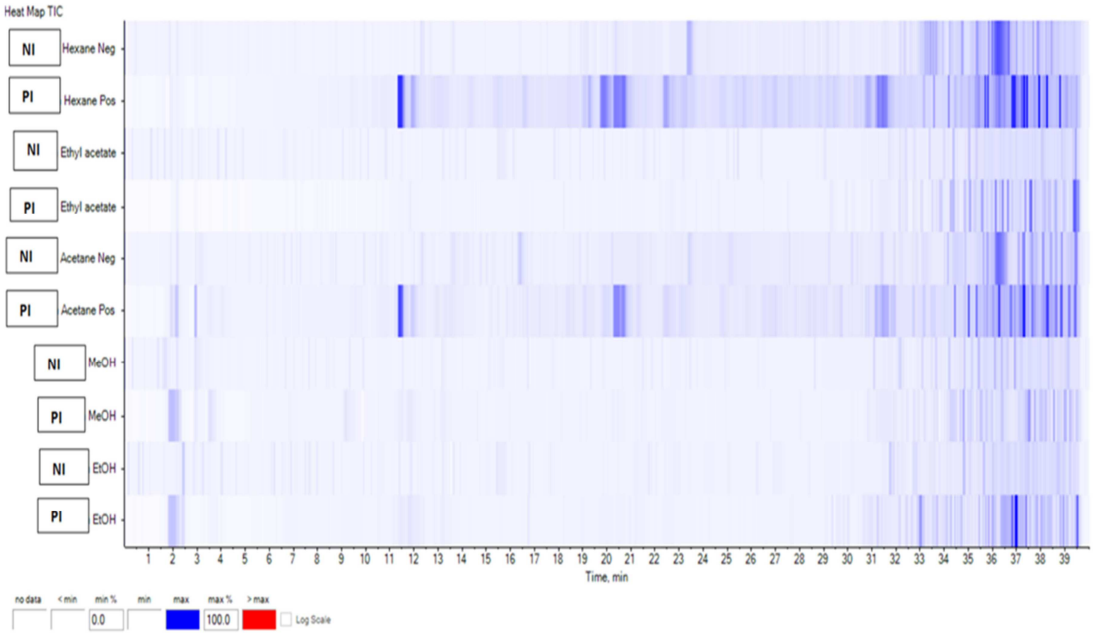
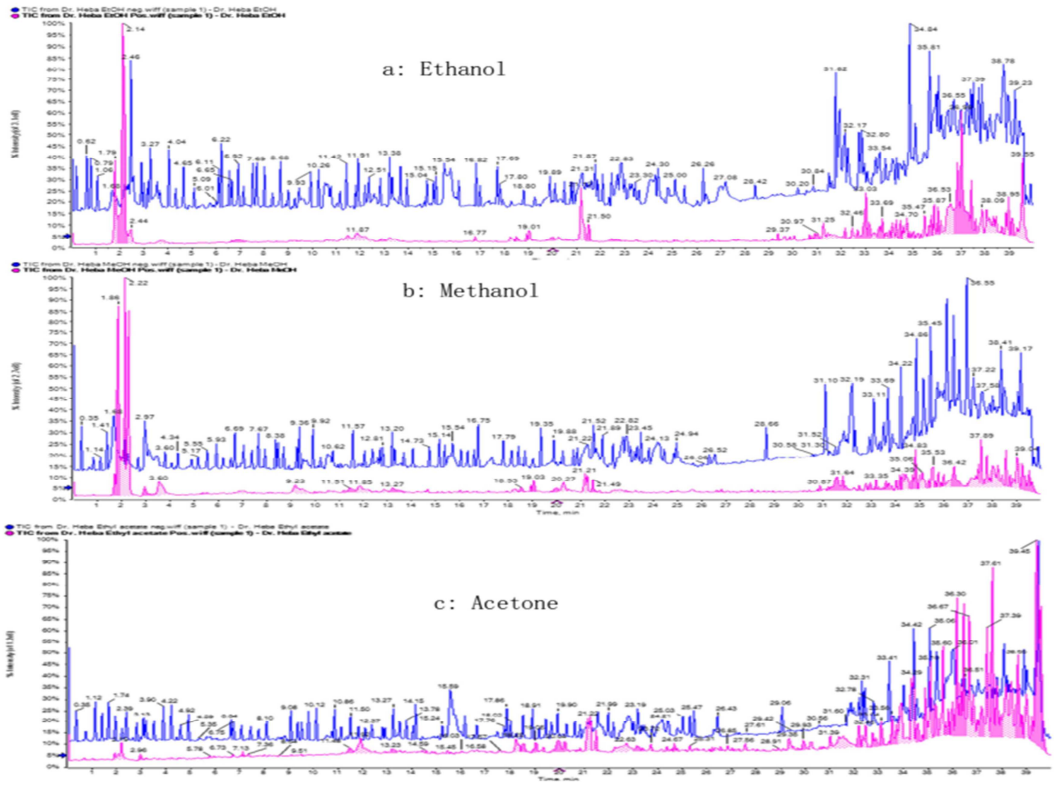



**Figure 1:** Combined Heatmap of *Chenopodium murale* five crude extracts in PI and NI. Blue lines represent metabolites. A dense blue colour means more or higher Ion intensity is present. The data results for the five extracts with the PI and NI ionization mode are collectively represented by heatmaps in one single pane for better comparison using PeakView® 1.2.0.3. Software of AB-SCIEX®. Copyright 2012 AB SCIEX



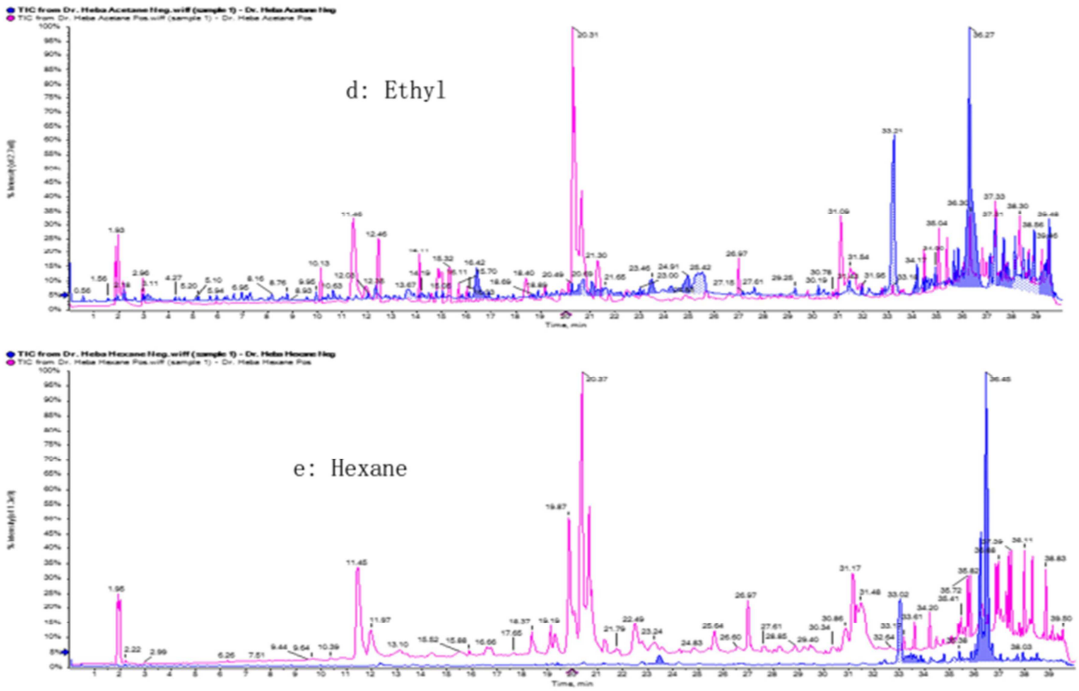



**Figure 2:** TIC chromatogram of *C. murale* five crude extracts [a-e] in positive ionization mode PI [TIC chromatogram peak line in red] & negative ionization mode NI [TIC chromatogram, peak line in blue]

### 3.2 Cytotoxicity test

Cell viability assay by the trypan blue dye exclusion method [15] on Hep-2 and BGM cell cultures with the acetone, hexane, ethyl acetate, methanol, and ethanol extracts of the *C. murale* plant was done, and Viable cells were morphologically examined and counted under the phase contrast microscope (Table 1).

Nontoxic doses of BGM and Hep-2 cells for acetone, hexane, ethyl acetate, methanol, and ethanol extracts of the *C. murale* were recorded in Table 2. Maximum toxicity was recorded with methanol and hexane while moderate to low with the alcohol, ethyl acetate, and acetone toward both cell lines

**Table 1: Cell viability assay results and the Nontoxic doses of tested materials on BGM and Hep-2 cell lines**

| Tested compounds | Nontoxic doses of BGM (µg/mL) | Nontoxic doses of Hep-2 (µg/mL) |
|---|---|---|
| Acetone | 28 | 29 |
| Hexan | 22 | 24 |
| Ethyl_acetate | 27 | 29 |
| Methanol | 20 | 21 |
| Ethanol | 26 | 26 |

### 3.3 Antiviral activity

#### 3.3.1 Quantification of infectious bacteriophage phiX174 virus

*C. murale* crude extracts of acetone, ethyl acetate, methanol, and ethanol have promising antiviral capabilities against bacteriophage phiX174 virus [three levels viral titers]. Maximum reduction was recorded by ethanol and methanol. Unlikely hexane samples demonstrated no antiviral effect against bacteriophage phiX174 (Table 2).

**Table 2: Reduction of bacteriophage phiX174 virus**

| Tested compounds | Initial viral titer | Final viral titer | % of reduction | Mean % of reduction |
|---|---|---|---|---|
| Acetone | $5X10^7$ | $2X10^7$ | 60% | 53.33% |
| | $5X10^6$ | $2X10^6$ | 60% | |
| | $5X10^5$ | $3X10^5$ | 40% | |
| Hexan | $5X10^7$ | $5X10^7$ | 0% | 0% |
| | $5X10^6$ | $5X10^6$ | 0% | |
| | $5X10^5$ | $5X10^5$ | 0% | |
| Ethyl acetate | $5X10^7$ | $1X10^7$ | 80% | 73% |
| | $5X10^6$ | $1X10^6$ | 80% | |
| | $5X10^5$ | $2X10^5$ | 60% | |
| Methanol | $5X10^7$ | $5X10^6$ | 90% | 90% |
| | $5X10^6$ | $5X10^5$ | 90% | |
| | $5X10^5$ | $5X10^4$ | 90% | |
| Ethanol | $5X10^7$ | $5X10^6$ | 90% | 90% |
| | $5X10^6$ | $5X10^5$ | 90% | |
| | $5X10^5$ | $5X10^4$ | 90% | |

Initial and final viral titer values are expressed as PFU/mL.

### 3.3.2 Determination of adenovirus type 7 and Coxsackievirus B4

*C. murale* crude methanol and ethanol extracts showed promising antiviral capability against Coxsackievirus B4 with 90%reduction (Table 3). Also, for the adenovirus type7, a similar record was achieved by methanol and ethanol, demonstrating a promising antiviral capability with 90% (Table 4). Unfortunately, the hexane extract had no antiviral effect against Coxsackievirus B4 and adenovirus type 7.

**Table 3: Reduction of Coxsackievirus B4**

| Tested compounds | Initial viral titer | Final viral titer | % of reduction | Mean % of reduction |
|---|---|---|---|---|
| Acetone | $5X10^5$ | $2X10^5$ | 60% | 46% |
| | $5X10^4$ | $3X10^4$ | 40% | |
| | $5X10^3$ | $3X10^3$ | 40% | |
| Hexan | $5X10^5$ | $4X10^5$ | 20% | 6% |
| | $5X10^4$ | $5X10^4$ | 0% | |
| | $5X10^3$ | $5X10^3$ | 0% | |
| Ethyl acetate | $5X10^5$ | $1X10^5$ | 80% | 66% |
| | $5X10^4$ | $2X10^4$ | 60% | |
| | $5X10^3$ | $2X10^3$ | 60% | |
| Methanol | $5X10^5$ | $5X10^4$ | 90% | 90% |
| | $5X10^4$ | $5X10^3$ | 90% | |
| | $5X10^3$ | $5X10^2$ | 90% | |
| Ethanol | $5X10^5$ | $5X10^4$ | 90% | 90% |
| | $5X10^4$ | $5X10^3$ | 90% | |
| | $5X10^3$ | $5X10^2$ | 90% | |

Initial and final viral titer values are expressed as PFU/mL.

**Table 4: Reduction of adenovirus type7**

| Tested compounds | initial viral titer | Final viral titer | % of reduction | Mean % of reduction |
|---|---|---|---|---|
| Acetone | $5X10^5$ | $3X10^5$ | 40% | 40% |
| | $5X10^4$ | $3X10^4$ | 40% | |
| | $5X10^3$ | $3X10^3$ | 40% | |
| Hexan | $5X10^5$ | $5X10^5$ | 0% | 0% |
| | $5X10^4$ | $5X10^4$ | 0% | |
| | $5X10^3$ | $5X10^3$ | 0% | |
| Ethyl acetate | $5X10^5$ | $2X10^5$ | 60% | 53% |
| | $5X10^4$ | $2X10^4$ | 60% | |
| | $5X10^3$ | $3X10^3$ | 40% | |
| Methanol | $5X10^5$ | $5X10^4$ | 90% | 86% |
| | $5X10^4$ | $5X10^3$ | 90% | |
| | $5X10^3$ | $1X10^3$ | 80% | |
| Ethanol | $5X10^5$ | $5X10^4$ | 90% | 90% |
| | $5X10^4$ | $5X10^3$ | 90% | |
| | $5X10^3$ | $5X10^2$ | 90% | |

Initial and final viral titer values are expressed as PFU/mL.

### 3.4 Antimicrobial activity

#### 3.4.1 Antimicrobial activities of *C. mural* crude extracts

C. mural crude extracts (acetone, ethanol, ethyl acetate, hexane, and methanol) were tested for their antimicrobial activities against different test organisms. The antimicrobial activity of the five extracts showed low inhibition zones (1-3 mm in diameter) when tested against microbial strains such as Bacillus cereus, Enterobacter faecalis, M. smegmatis, P. aeruginosa, Staphylococcus aureus in Table. 5, Aspergillus flavus, A. niger, A. sydowii, A. ustus, Candida albicans, and Saccharomyces cerevisiae in Table 6.

#### 3.4.2. Enhancement of the antimicrobial activities of *C. mural crude* extracts by mixing with herbal essential oils

*C. mural* crude extracts were combined with essential oils (Alo Vera, Cinnamon, Clove, Lavender, Lemon, Neem, Peppermint, and Green Tea) to enhance their antimicrobial activities. 2.5 µL of 200 µg/mL of different extracts were mixed with 2.5 µL of the oils.

Ethyl acetate-peppermint and Ethyl acetate-lemon mixtures showed antibacterial and antifungal activity (Table 7). The Ethyl acetate-**peppermint** mixture attained maximum inhibition (4 mm) against Aspergillus ustus.

Six methanol mixtures recorded antimicrobial activity (Table 8). The maximum inhibition (4 mm) was attained in the methanol-peppermint mixture against *Aspergillus niger, A. sydowii,* and *Candida albicans.*

Four acetone mixtures recorded both antibacterial and antifungal activities (Table 9). The maximum inhibition (3 mm) was recorded in the acetone-peppermint mixture against *Aspergillus flavus*, *A. sydowii,* and *Saccharomyces cerevisiae.*

Five ethanol mixtures recorded both antibacterial and antifungal activity (Table 10). The maximum inhibition (4 mm) was attained in the case of ethanol-lemon and ethanol-peppermint against *Aspergillus ustus*.

Seven Hexane mixtures recorded both antibacterial and antifungal activities (Table 11). The maximum inhibition (4 mm) was found in the case of Hexane-clove, Hexane-lemon, and hexane-peppermint against *Bacillus cereus, Aspergillus flavus,* and *Pseudomonas aeruginosa.* Minimum inhibitory concentration (MIC) was carried out through the dilution method. The minimum inhibitory concentration of all tested *Chenopodium* extracts was more significant at 100 µg/mL.

**Table 5: Antimicrobial activities of different crude extracts of *C. murale***

| Bacterial species | Inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Ciprofloxacin (control) | Acetone | Ethanol | Ethyl acetate | Hexane | Methanol |
| *Bacillus cereus* | 29 | 0 | 0 | 0 | 0 | 0 |
| *Enterobacter faecalis* | 34 | 0 | 0 | 4 | 1 | 0 |
| *Mycobacterium smegmatis* | 23 | 0 | 0 | 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | 34 | 2 | 2 | 0 | 0 | 1 |
| *Staphylococcus aureus* | 34 | 1 | 0 | 0 | 0 | 0 |

**Table 6: Antimicrobial activity of different crude extracts of *C. murale***

| Fungal species | Inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Tioconazole (control) | Acetone | Ethanol | Ethyl acetate | Hexane | Methanol |
| *Aspergillus flavus* | 9 | 0 | 0 | 0 | 0 | 0 |
| *A. niger* | 8 | 0 | 0 | 0 | 0 | 0 |
| *A. sydowii* | 8 | 0 | 1 | 0 | 0 | 0 |
| *A. ustus* | 7 | 0 | 0 | 1 | 0 | 0 |
| *Candida albicans* | 8 | 0 | 0 | 0 | 1 | 0 |
| *Saccharomyces cerevisiae* | 9 | 2 | 0 | 0 | 0 | 1 |

**Table 7: Antimicrobial activity of ethyl acetate extract of *C. murale* mixed with different essential oils**

| Microorganisms | Inhibition zone diameter (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Aloe vera | Cinnamon | Clove | Lavender | Lemon | Neem | Peppermint | Green Tea |
| *Bacillus cereus* | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Enterobacter* | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Pseudomonas aeruginosa* | 22 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Mycobacterium* | 25 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 |
| *Pseudomonas aeruginosa* | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus flavus* | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| *A. niger* | 10 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| *A. sydowii* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus ustus* | 9 | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 0 |
| *Candida albicans* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| *Saccharomyces cerevisiae* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Control: (fungi) Tioconazole; (Bacteria) Ciprofloxacin
Green tea comprises catechins, caffeine, theanine, flavonoids, minerals, vitamins c and b, and volatile compounds such as aldehydes, alcohols, esters, and terpenes.

**Table 8: Antimicrobial activity of Methanol extract of *C. murale* mixed with different essential oils**

| Microorganisms | Inhibition zone diameter (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Aloe vera | Cinnamon | Clove | Lavender | Lemon | Neem | Peppermint | Neem | Green Tea |
| *Bacillus cereus* | 23 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 5 | 0 |
| *Enterobacter* | 25 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | 24 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 1 |
| *Mycobacterium* | 22 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| *Staphylococcus aureus* | 21 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 2 | 1 |
| *Aspergillus flavus* | 9 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus niger* | 8 | 0 | 0 | 0 | 0 | 2 | 3 | 4 | 3 | 0 |
| *A. sydowii* | 7 | 0 | 2 | 0 | 0 | 3 | 3 | 4 | 3 | 1 |
| *A. ustus* | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Candida albicans* | 11 | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| *Saccharomyces cerevisiae* | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Control: (fungi) Tioconazole; (Bacteria) Ciprofloxacin

**Table 9: Antimicrobial activity of acetone extract of *C. murale* mixed with different essential oils**

| Microorganisms | Inhibition zone diameter (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Aloe vera | Cinnamon | Clove | Lavender | Lemon | Neem | Peppermint | Green Tea |
| *Bacillus cereus* | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Enterobacter* | 23 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 |
| *Pseudomonas aeruginosa* | 23 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 1 |
| *Mycobacterium* | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S. aureus* | 22 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| *Aspergillus flavus* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| *A. niger* | 8 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| *A. sydowii* | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| *A. ustus* | 9 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Candida albicans* | 7 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Saccharomyces cerevisiae* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |

Control: (fungi) Tioconazole; (Bacteria) Ciprofloxacin

**Table 10: Antimicrobial activity of ethanol extract of *C. mural* mixed with different essential oils**

| Microorganisms | Inhibition zone diameter (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Aloe vera | Cinnamon | Clove | Lavender | Lemon | Neem | Peppermint | Green Tea |
| *Bacillus cereus* | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Enterobacter* | 23 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 |
| *Pseudomonas aeruginosa* | 26 | 0 | 1 | 0 | 1 | 2 | 0 | 2 | 0 |
| *Mycobacterium* | 24 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *S. aureus* | 24 | 0 | 1 | 0 | 0 | 2 | 0 | 3 | 0 |
| *Aspergillus flavus* | 8 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *A. niger* | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| *A. sydowii* | 8 | 0 | 1 | 0 | 1 | 3 | 0 | 0 | 2 |
| *A. ustus* | 7 | 0 | 0 | 0 | 0 | 4 | 0 | 4 | 0 |
| *Candida albicans* | 8 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Saccharomyces cerevisiae* | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Control: (fungi) Tioconazole; (Bacteria) Ciprofloxacin

**Table 11: Antimicrobial activity of hexane extract of *C. murale* mixed with different essential oils**

| Microorganisms | Inhibition zone diameter (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Aloe vera | Cinnamon | Clove | Lavender | Lemon | Neem | Peppermint | Green Tea |
| *Bacillus cereus* | 22 | 0 | 3 | 4 | 0 | 3 | 0 | 4 | 0 |
| *Enterobacter* | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pseudomonas aeruginosa* | 22 | 1 | 3 | 0 | 0 | 4 | 0 | 2 | 3 |
| *Mycobacterium* | 24 | 1 | 2 | 1 | 1 | 1 | 0 | 2 | 1 |
| *s. aureus* | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus flavus* | 8 | 0 | 3 | 4 | 1 | 2 | 0 | 4 | 3 |
| *A. niger* | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. sydowii* | 8 | 0 | 2 | 2 | 0 | 1 | 0 | 2 | 3 |
| *A. ustus* | 7 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Candida albicans* | 8 | 0 | 3 | 1 | 1 | 1 | 0 | 2 | 2 |
| *Saccharomyces cerevisiae* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Control: (fungi) Tioconazole; (Bacteria) Ciprofloxacin

## 4. Discussion

Plants have been used as a source of nutrients and remedies since human colonisation on Earth. This appeared in every human culture worldwide as herbal medicine, which plays a significant role in public health because of its natural richness and availability as fresh green vegetables or extracts. Plant extracts as a sustainable resource attract drug discovery and the pharmaceutical industries for combating and defeating outbreaks and emerging new or eradicated diseases. The coronavirus COVID-19 pandemic woke the world with an urgent need for new antiviral agents to fill the gap of no cure. This attracted us to the *Chenopodium murale* plant, a useless weed in many crops commonly found in Egypt but very rich in nutraceuticals for medical or health benefits. This study targeted the antiviral activity and cytotoxicity with a complete putative metabolite profile for the most potent extract. The two tested cell lines (BGM and Hep-2) showed no significant difference in sensitivity to toxic metabolites' content of the five *C. murle* crude extracts. A promising antiviral activity recording the efficacy of the crude ethanol extract against the tested viruses and, more specifically, towards adenovirus type 7 with a 90 % mean reduction in viral titer over all tested extracts. Moreover, the two tested cell lines (BGM and Hep-2) showed no significant toxicity toward the metabolites' content of the *C. murle* crude extracts.

Concluding that the high sensitivity of QTRAP®with a **LIT** technology with EMS and EPI scans for screening, confirmation, and identification for both PI and NI modes significantly revealed the rich content of compounds in the *C. murale* crude ethanolic extract as summarised in **Table 12,** which was in agreement with what was declared by [1,17,46,50,85,86,87]

Adenovirus types 3, 4, and 7 are most commonly associated with acute respiratory illness. The Adenovirus type7 has been associated with more severe outcomes than other types, particularly in people with weakened immune systems. In the last 10 years, severe illness and death from adenovirus type 7 infection have been reported in the United States, as reported by the CDC in 2022.

For antimicrobial activity, Several reports showed that the essential oil of *C. murale* is rich in p-cymene and a-terpinene [89-91] [97-99] , which are related to its medicinal applications. Various plant extracts from traditional medicinal plants were assayed to determine new sources of therapeutic importance. *C. mural* was proven to be active against different microbial species. The extracts were combined with different essential oils (Alo Vera, Cinnamon, Clove, Lavender, Lemon, Neem, Peppermint, and Tea) to increase their antimicrobial activity.

The results were in good accordance with the findings of Parkash *et al.* (2015), Kaur *et al*. (2018), and Külcü *et al*. (2019) [92-94]. They stated that the Ethyl acetate extract of *Chenopodium* was promising in inhibiting *Staphylococcus aureus*, *E. coli,* and *Lactobacillus* and presented the lowest MIC values against *Staphylococcus aureus* and *Enterococcus faecalis Pseudomonas aeruginosa*, *Paenibacillus apiarus* and *Paenibacillus thiaminolyticus* [95].

Plants have been used as a source of nutrients and remedies since human colonisation on Earth. This appeared in every human culture worldwide as herbal medicine, which plays a significant role in public health because of its natural richness and availability as fresh green vegetables or extracts. Plant extracts as a sustainable resource

attract drug discovery and the pharmaceutical industries for combating and defeating outbreaks of emerging new or eradicated diseases. The coronavirus COVID-19 pandemic woke the world with an urgent need for new antiviral agents to fill the gap of no cure. This attracted us to the *Chenopodium murale* plant, a useless weed in many crops commonly found in Egypt but very rich in nutraceuticals for medical or health benefits. This study targeted the antiviral activity and cytotoxicity with a complete putative metabolite profile for the most potent extract. The two tested cell lines (BGM and Hep-2) showed no significant difference in sensitivity or toxicity towards the metabolites' content of the five *C. murle* crude extracts. A promising antiviral activity recording the efficacy of the crude ethanol extract against the tested viruses and, more specifically, towards adenovirus type 7 with a 90 % mean reduction in viral titer over all tested extracts.

The results were in good accordance with the findings of Parkash *et al.* (2015), Kaur *et al*. (2018), and Külcü *et al*. (2019)[96-99].

The results suggest the possible use of the *Chenopodium mural* due to its antimicrobial activity against different bacterial and fungal species.

**Table 12: Chemical classes of putatively identified compounds with reported antiviral and biological activities**

| Chemical class | Compound name | *Compound No. & Supported References |
|---|---|---|
| 1-pentoses, hexoses, and derivatives | Fructose-1,6-bisphosphate, 2-deoxyribose, Arabinose, Glyceric acid | [22, 25, 28, 47, 78] |
| 2- amino acids and derivatives | Guanidinoacetic acid, Valine, N-Acetylglycine, Phenylalanine, N-Methylserine, Pyroglutamic acid, Homoserine, Glutamic acid, Ornithine, 5-aminovaleric acid, N-Acetylcysteine, gamma-Glutamyl-cysteine, | [20, 19, 26, 31, 32, 33, 35, 49, 52, 58, 98] |
| 3- Nonpolar constituents and derivatives | Docosatetraenoic acid, Palmitoylcarnitine cation, Palmitic acid, Arachidic acid, Heneicosanoic acid, Methyl Heptadecanoic acid, Octadecanedioic acid, Oleic acid, Palmitoleic acid, Lauric acid, Ethyl myristate | [1, 44, 45, 53, 61, 70, 76, 77] |
| 4- Carboxylic acid and derivatives (Pyrimidine, Pyridine | Chlorogenic acid, Quinic acid, Jasmonic acid, Pyrrole-2-carboxylic acid, Nicotinic acid, Urocanic acid, Citric acid, Nialamide, Ketoisovaleric acid. | [2, 11, 16, 27, 28, 64] |
| Flavonoids | Kaempferol, Luteolin, Glycitein, Genistein, Equol, Isorhamnetin, Ipriflavone, Luteolin-6-C-glucoside, Vitexin, Riboflavin, Nobiletin | [5, 44, 93, 54, 60, 74, 75, 82] |
| Glycosides | trans-Piceid, Glucobrassicin, Naringin, Geniposide, Luteolin-6-C-glucoside, Piceid | [84, 93, 95] |
| Nucleoside and nucleotide analogs | Cytarabine, Cladribine, 5-Hydroxymethylcytidine, 1-Methylpseudouridine, Xanthosine, 2'-Deoxyuridine 5'-monophosphate, N-Acetylcytidine, Guanosine 5'-monophosphate, Cytidine-5'-monophosphate, 2'-O-Methyl-5-methylcytidine, Inosine-5-monophosphate, 5'-Methylthioadenosine, 2'-Deoxyguanosine, Guanosine, Cordycepin, 2'-O-Methylinosine, S-Adenosyl-methionine, 1-Methyladenosine, N6-Isopentenyladenosine[Riboprine], 2,3-Dideoxyuridine. | [23, 35, 36, 42, 43, 46, 83, 55, 57, 59, 62, 63, 66, 79, 80, 91, 93, 94] |
| Phenols | 2-Aminophenol, 5-Hydroxytryptophan, Phloretin, Dihydrocapsaicin, Capsaicin, Salicin, Coniferylaldehyd, 4-Hydroxyphenylpyruvic acid, Acetaminophen glucuronide, Chlorogenic acid, Tremulacin | [5, 17, 21, 29, 30, 37, 41, 42, 48, 73, 82] |
| Carotenoid Terpenoids, sterols | Cocaethylene, Limonene, Thymol, Chlorogenic acid, Salmeterol, Geranic acid, 5-Cholesten-3-beta-7-alpha-diol, sitosterols. | [38, 68, 81, 83, 88] |
| Alkaloids | Synephrine, Ecgonine, Lomifylline, Rinderine, 1,7-Dimethylxanthine, Brucine, CHEMBL1616521-a morphinane alkaloid. | [39, 54, 65, 69, 72, 81, 84] |
| Amides | Choline [M]+, Actrarit, salicylamides, Cinanserin | [4, 24, 56, 67] |
| Vitamin | Retinoic acid * | [42, 53, 73] |
| Peptides | Carnosine | [34, 92] |

* **Compound number as listed in Supplementary data for more details**

## 5. Conclusion

Overall, the results of this study suggest that Chenopodium murale holds significant potential as a source of medicinal agents. Its extracts demonstrated notable antiviral activity, particularly against adenovirus type 7, a common cause of respiratory illness. In addition, the extracts showed antimicrobial activity against various bacterial and fungal species. These findings indicate that C. murale could be valuable in developing new antiviral and antimicrobial treatments. Further research is needed to fully understand these activities' mechanisms and explore the potential for clinical applications.

## 6- Declarations

**Ethics approval and consent to participate:**

A fresh *Chenopodium murale* plant sample was obtained from cultivation sites in Egypt in February 2021 after receiving the required permissions from the owners.

Taxonomic identification of the fresh *C. murale* plant was generously identified and confirmed by the senior taxonomist at Cairo University Herbarium and has already been registered.

**Consent for publication:** Not applicable

**Availability of data and materials:** The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

**Conflict of Interest:** The authors declare no conflict of interest.

**Funding:** This work was funded by Cairo University

**Author's Contribution:** " Gihan M. Zaghlol, Heba M. Fahmy, and Mohsen A. Sayed contributed to the study conception and design. Sayed M.S. Abo El-Souad, Amena S. El-Feky, Sohaila S. Awad, Fatma R. Elfiky, Maram A. Abdelghany, and Hanaa H.B. Elmalla helped with materials, sample collection, Preparation, and initial data collection. Mohsen A. Sayed and M.S. Abo El-Souad made the practical microbiology work, Microbiology data analysis. LC-MS/MS, antiviral and cytotoxicity data analyses, and the first and final draft of the manuscript were performed by Gihan M. Zaghlol. Heba M. Fahmy Received the funds from Cairo University, supervised the project, and participated in revising the first draft. All authors read and approved the final manuscript."

**Acknowledgement**

The authors thank **Cairo University** for funding and supporting this project as part of the Research Call to combat COVID-19.

## 7. References

1) Naqvi SF, Javaid A, Qureshi MZ. Evaluation of antifungal potential of leaf extract of Chenopodium murale against fusarium oxysporum f. sp. lycopersici. Planta daninha.2019 ;37 doi:10.1590/S0100-83582019370100139
2) Şimşek Sezer EN, Uysal, T. Phenolic screening and biological activities of Chenopodium botrys L. extracts. Ant J Bot. 2021.
3) Mohasib RM, Nagib A, Samad AA, Salama ZA, Gaafar AA, Taie HA, Hussein SR. Identification of bioactive ingredients in Chenopodium murale L Chenopodiaceae by HPLC and GC/MS. Research RJPT. 2022;15(1): 177-187.
4) da Silva JKR, Figueiredo PLB, Byler KG, Setzer WN. Essential oils as antiviral agents, potential of essential oils to treat SARS-CoV-2 infection: An in-silico investigation. Int. J. Mol. Sci., 2020;21(10): 3426.
5) Poonia A. Bioactive Compounds of Fat-Hen (Chenopodium album L.). In: Murthy, H.N, Paek, K.Y. (eds) Bioactive Compounds in Underutilised Vegetables and Legumes. Reference Series in Phytochemistry. Springer, Cham. 2021; https://doi.org/10.1007/978-3-030-57415-4_6
6) Sykłowska-Baranek, K.; Gaweł, M.; Kuźma, Ł.; Wileńska, B.; Kawka, M.; Jeziorek, M.; Graikou, K.; Chinou, I.; Szyszko, E.; Stępień, P.; et al. *Rindera graeca* (A. DC.) Boiss. & Heldr. (Boraginaceae) In Vitro Cultures Targeting Lithospermic Acid B and Rosmarinic Acid Production. *Molecules* **2023**, *28*, 4880. https://doi.org/10.3390/molecules28124880
7) Mtenga, Deodata V, and Asha S Ripanda. "A review on the potential of underutilised Blackjack *(Biden Pilosa)* naturally occurring in sub-Saharan Africa." *Heliyon* vol. 8,6 e09586. 29 May. 2022, doi:10.1016/j.heliyon.2022.e09586
8) Khan N, Ahmed M, Khan RA, Gul S. Antioxidant, cytotoxicity activities, and phytochemical analysis of Chenopodium murale (Linn.). Int. J. Bot. 2019;4:25-28.
9) Ghareib HRA, Abdelhamed MS, Ibrahim OH. Antioxidative effects of the acetone fraction and vanillic acid from Chenopodium murale on tomato plants. Weed Biol. 2010;10(1): 64-72.
10) Adedapo A, Jimoh, F, Afolayan A. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of Bidens pilosa and Chenopodium album. Acta Pol Pharm.2011; 68(1): 83-92.
11) Javaid A, Amin M. Antifungal activity of methanol and n-hexane extracts of three Chenopodium species against Macrophomina phaseolina. Nat. Prod. Res..2009;23(12):1120-1127.
12) Naqvi SF, Khan IH, Javaid A. Hexane soluble bioactive components of Chenopodium murale stem. J. Weed Sci. Res. 2020;26(4):425.
13) Javaid A, Naqvi SF, Khan IH. Ethyl Acetate Extract of Chenopodium murale Root, a Source of Bioactive Compounds. J. Weed Sci. Res. 2021; 27(1):93.

14) Simo˜es CMO, Amoros M, L. Girre polis S. Mechanism of Antiviral Activity of. Triterpenoid Saponins. Phytother. Res.1999;13: 323–328.
15) Walum E, Stenberg K, Jenssen D. Understanding Cell Toxicology: principles and practice. Ellis Howood, NewYork.1990; 97-111.
16) Baird R, Bridgewater L. (Ed.) . Standard methods for the examination of water and wastewater (23rd ed.): Washington D.C,2017.
17) Schmidtke M, Knorre C, Blei L, Stelzner A, Birch-Hirschfeld E. Penetration and Antiviral Activity of Coxsackievirus B3 (Cvb3)- Specific Phosphorothioate Oligodeoxynucleotides (Ps-Odn). Nucleosides and Nucleotides. 1998; 17: 1557-1566.
18) Wang GF, Shi LP, Ren YD, Liu QF, Liu HF, Zhang RJ, Zuo JP. Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. Antiviral Res.2009;83(2):186-190.
19) Civra A, Francese R, Sinato D, Donalisio M, Cagno V, Rubiolo P, Lembo D. In vitro screening for antiviral activity of Turkish plants revealing methanolic extract of Rindera lanata var. lanata active against human rotavirus. BMC Complement Altern Med. 2017;17(1):74.
20) Hafiz TA, Mubaraki MA, Dkhil MAM, Al-Quraishy S. Antiviral Activities of Capsicum annuum Methanolic Extract against Herpes Simplex Virus 1 and 2. Pakistan J. Zool. 2017;49:251-255.
21) KaraÇElİK AA, ŞAhİN H. Determination of Chemical Compositions, Antioxidant and Enzyme Inhibitory Activities of Naturally Growing Chenopodium album subsp. iranicum Aellen. JIST.2021;2091-2101. doi:10.21597/jist.825781
22) Chakraborty K, Chakravarti, A. R, and Bhattacharjee, S. Bioactive components of peppermint (Mentha piperita L.), their pharmacological and ameliorative potential and ethnomedicinal benefits: A review. RJPP. 2022;11(1):109-114**.**
23) Rakedzon S, Neuberger A, Domb AJ, Petersiel N, Schwartz E. From hydroxychloroquine to ivermectin: what are the antiviral properties of anti-parasitic drugs to combat SARS-CoV-2?. J. Travel Med. 2021;28(2): taab005.
24) Ghiasifar Z, Salehabadi H, Adibpour N, Alipour E, Kobarfard F, Shoushizadeh MR. Synthesis of Biuret Derivatives as Potential HIV-1 Protease Inhibitors Using (LDHs-g-HMDI-Citric Acid), as a Green Recyclable Catalyst. BKCS.2021; 42(1): 48-59.
25) Li J, Jiang X, Dick A, Sharma PP, Chen CH, Rathi B, Zhan, P. Design, synthesis, and antiviral activity of phenylalanine derivatives as HIV-1 capsid inhibitors. Bioorganic Med. Chem. Lett. 2021;48:116414.
26) Melano I, Kuo LL, Lo YC, Sung PW, Tien N, Su WC. Effects of Basic Amino Acids and Their Derivatives on SARS-CoV-2 and Influenza-A Virus Infection. Viruses. Jul 4 2021;13(7):1301. doi: 10.3390/v13071301. PMID: 34372507; PMCID: PMC8310019
27) Cook GA, Dawson LA, Tian Y, Opella, SJ. Three-dimensional structure and interaction studies of hepatitis C virus p7 in 1, 2-dihexanoyl-sn-glycero-3-phosphocholine by solution nuclear magnetic resonance. Biochem., 2013;52(31):5295-5303.
28) Ajaib M, Hussain T, Farooq S, Ashiq M. Analysis of Antimicrobial and Antioxidant Activities of Chenopodium ambrosioides An Ethnomedicinal Plant. J. Chem., 2016
29) Curreli F, Belov DS, Ramesh RR, Patel N, Altieri A, Kurkin A. V, and Debnath, A. K. Design, synthesis and evaluation of small molecule CD4-mimics as entry inhibitors possessing broad spectrum anti-HIV-1 activity. Bioorganic Med. Chem. Lett.2016;24(22): 5988-6003.
30) Ogasawara Y, Dairi T. Biosynthesis of oligopeptides using ATP-grasp enzymes. CHEM-EUR J.2017; 23(45):10714-10724.
31) Dar-Odeh N, Elsayed S, Babkair H, Abu-Hammad S, Althagafi N, Bahabri R, Abu-Hammad O. What the dental practitioner needs to know about pharmaco-therapeutic modalities of COVID-19 treatment: A review. J. Dent. Sci..2021; 16(3): 806-816.
32) Zhou D, Xie D, He F, Song B, Hu D. Antiviral properties and interaction of novel chalcone derivatives containing a purine and benzenesulfonamide moiety. Bioorganic Med. Chem. Lett..2018; 28(11):2091-2097.
33) Cordt C, Meckel T, Geissler A, Biesalski M. Entrapment of hydrophobic biocides into cellulose acetate nanoparticles by nanoprecipitation. Nanomater.2020; 10(12):2447.
34) Asiri YI, Alsayari A, Muhsinah AB, Mabkhot YN, Hassan MZ. Benzothiazoles as potential antiviral agents. J. Pharm. Pharmacol. 2020;72(11): 1459-1480.
35) Singh B. Effect of antiviral chemicals on in vitro regeneration response and production of PLRV-free plants of potato. JCSB. 2015;18(5): 341-348.
36) Skwarecki AS, Nowak MG, Milewska MJ. Amino Acid and Peptide-Based Antiviral Agents. ChemMedChem. 2021;16(20): 3106-3135.
37) Narang R, Narasimhan B, Sharma S, Sriram D, Yogeeswari P, Clercq ED, Balzarini, J . Nicotinic acid benzylidene/phenyl-ethylidene hydrazides: synthesis, antimicrobial evaluation and QSAR studies. Lett. Drug Des. Discov. 2011;8(8):733-749.
38) Tayebania M, Alizadeh R. Synthesis and characterisation of transition metal complexes with Terphthalaldehyde based bidentate Schiff base ligand and catalytic activity of aromatic alcohol oxidation.2014.
39) Medina JJ M, Naso LG, Pérez AL, Rizzi A, Okulik NB, Valcarcel M, Williams PA. Synthesis, characterisation, theoretical studies and biological (antioxidant, anticancer, toxicity and neuroprotective) determinations of a copper (II) complex with 5-hydroxytryptophan. Biomed. Pharmacother.2019;111: 414-426.
40) Sun L, Dick A, Meuser M.E, Huang T, Zalloum WA, Chen CH, Zhan P. Design, synthesis, and mechanism study of benzenesulfonamide-containing phenylalanine derivatives as novel HIV-1 capsid inhibitors with improved antiviral activities. J. Med. Chem..2020;63(9): 4790-4810.
41) Lin SC, Chen MC, Liu S, Callahan VM, Bracci NR, Lehman CW, Kehn-Hall K. Phloretin inhibits Zika virus infection by interfering with cellular glucose utilisation. Int. J. Antimicrob. Agents.2019;54(1):80-84.

42) El-Sayed WA, Khalaf HS, Mohamed SF, Hussien HA, Kutkat OM, Amr AE. Synthesis and antiviral activity of 1, 2, 3-triazole glycosides based substituted pyridine via click cycloaddition. Russ. J. Gen. Chem..2017; 87(10): 2444-2453.
43) Townsend TM, Bernskoetter WH, Hazari N, Mercado BQ. Dehydrogenative Synthesis of Carbamates from Formamides and Alcohols Using a Pincer-Supported Iron Catalyst. ACS Catalysis.2021; 11(16): 10614-10624.
44) Oien NL, Brideau R.J, Hopkins TA, Wieber JL, Knechtel ML, Shelly JA, Wathen MW. Broad-spectrum antiherpes activities of 4-hydroxyquinoline carboxamides, a novel class of herpesvirus polymerase inhibitors. Antimicrob. Agents Chemother. 2002; 46(3): 724-730.
45) Sobala LF, Fernandes PZ, Hakki Z, Thompson AJ, Howe JD, Hill M, Davies GJ. Structure of human endo-a-1,2-mannosidase (MANEA), an antiviral host-glycosylation target. PNAS.2020;117(47): 29595–29601.
46) Ding S, Greenberg HB. Zika mRNA vaccine induces long-term protective immunity. AME Medical Journal DOI,10.2017.
47) Rezazadeh A, Hamishehkar H, Ehsani A, Ghasempour Z, Moghaddas Kia E. Applications of capsaicin in food industry: functionality, utilisation and stabilisation. Crit. Rev. Food Sci. Nutr.. 2021:1-17.
48) Boris R, Elena T, Erich S, Walter J, Gerhard B, Leopold J. Cytotoxic Properties of Selected Sesquiterpene Alcohols on Human Cervix Carcinoma Cell Lines. J. Essent. Oil-Bear..2011; 14(3): 316-319.
49) Hussein YA, Sivakumar V, Elazazy M, Ganesh K. Synephrine as Antioxidant: Application in Quenching of Photo Induced Radical of Anthraquinone and Naphthoquinone. In Qatar Foundation Annual Research Conference Proceedings. Hamad bin Khalifa University Press (HBKU Press).1;2016:HBpp2023.
50) Vanderlinden E, Marchand A, Van Berwaer R, van Dam W, Arzel P, Klaassen H, Naesens L. A broad influenza virus inhibitor acting via IMP dehydrogenase and in synergism with ribavirin. Antivir. Res. 2021;196: 105208.
51) Morita T, Miyakawa K, Jeremiah SS, Yamaoka Y, Sada M, Kuniyoshi T, Yang J, Kimura H, Ryo A. All-Trans Retinoic Acid Exhibits Antiviral Effect against SARS-CoV-2 by Inhibiting 3CLpro Activity. Viruses.2021;13(8):1669. doi: 10.3390/v13081669. PMID: 34452533; PMCID: PMC8402917.
52) Smitha CK, Udayan PS, Vaishnavi M. Unraveling the Phytochemistry of Ethnomedicinal Tree Pajanelia longifolia (Willd.), Schum, K. In: Arunachalam, K, Yang, X, Puthanpura Sasidharan, S. (eds) Natural Product Experiments in Drug Discovery. Springer Protocols Handbooks. Humana, New York, NY.2023.
53) Hu Z, Zhou J, Han L, Li X, Li C, Wu T, Chen X. Acyclovir alleviates insulin resistance via activating PKM1 in diabetic mice. Lif. Sci., .2022; 304:120725.
54) Aghamohamadi N, Sanjani NS, Majidi RF, Nasrollahi SA . Preparation and characterisation of Aloe vera acetate and electrospinning fibers as promising antibacterial properties materials. MSEB., 2019; 94: 445-452.
55) Bösch NM, Borsa M, Greczmiel U, Morinaka BI, Gugger M, Oxenius A, Piel, J. Landornamides: antiviral ornithine-containing ribosomal peptides discovered through genome mining. Angewandte Chemie.2020; 132(29): 11861-11866.
56) Zhai X, Wang N, Jiao H, Zhang J, Li C, Ren W, Su S. Melatonin and other indoles show antiviral activities against swine coronaviruses in vitro at pharmacological concentrations. J. Pineal Res..2021; 71(2): e12754.
57) Almoalem M, AlAlharith I, Alomer H, Almarri A, Alyami A, Hamzah R, Alnoaimi S. Extensive eczema herpeticum in a previously well child. Int. J. Emerg. Med., .2022; 15(1): 1-3.
58) Yan H, Ma L, Wang H, Wu S, Huang H, Gu Z, Li Y. Luteolin decreases the yield of influenza A virus in vitro by interfering with the coat protein I complex expression. J. Nat. Med., 2019;73(3):487-496.
59) Ouyang Y, Wu Q, Li J, Sun S, Sun S. S-adenosylmethionine: a metabolite critical to the regulation of autophagy. Cell Prolif., 2020;53(11): e12891.
60) Moeller NH, Passow KT, Harki DA, Aihara H. SARS-CoV-2 nsp14 Exoribonuclease Removes the Natural Antiviral 3′-Deoxy-3′, 4′-didehydro-cytidine Nucleotide from RNA. Viruses. 2022;14:1790.
61) Verma AK.Cordycepin: a bioactive metabolite of C ordyceps militaris and polyadenylation inhibitor with therapeutic potential against COVID-19.J Biomol Struct Dyn.2022;40:3745-3752.
62) Gurusamy S, Kulanthaisamy MR, Hari DG, Veleeswaran A, Thulasinathan B, Muthuramalingam JB, Alagarsamy A. Environmental friendly synthesis of TiO2-ZnO nanocomposite catalyst and silver nanomaterials for the enhanced production of biodiesel from Ulva lactuca seaweed and potential antimicrobial properties against the microbial pathogens. J Photoch Photobio B. 2019;193:118-130.
63) Arribat M, Cavelier F, Rémond E. Phosphorus-containing amino acids with a P–C bond in the side chain or a P–O, P–S or P–N bond: from synthesis to applications. RSC Adv.2020;10:6678-6724.
64) Fu ZD, Selwyn FP, Cui JY, Klaassen CD. RNA-Seq profiling of intestinal expression of xenobiotic processing genes in germ-free mice. Drug Metab Dispos. 2017;45:1225-1238.
65) Qiu T, Wang J, Zhou J, Zou J, Chen Z, Ma X, Zhang L.The report of two cases infection with novel coronavirus (2019-ncov) after kidney transplantation and the association literature analysation. Chinese Journal of Organ Transplantation. 2020;E004-E004.
66) Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku F.GCMS analysis of phytocomponents in the methanolic extract of Moringa oleifera leave. Int J Pharm Sci Res.2015;2:1-6.
67) Li X, Yang Y, Liu L, Yang X, Zhao X, Li Y, ... Huang S.Effect of combination antiviral therapy on hematological profiles in 151 adults hospitalised with severe coronavirus disease 2019. Pharmacol Res.2020;160:105036.
68) D'Innella P, Zaccala G, Terazzi M, Olgiati P, Torre E.Protective effect of olanzapine in psychotic disorder induced by interferon-alpha. Recenti Progressi Med.2003;94:343–344.
69) Han YX, Xue R, Zhao W, Zhou ZX, Li JN, Chen HS, … Wu, YW. Antiviral therapeutic efficacy of foscarnet in hepatitis B virus infection. Antiviral Res.2005;68:147–153.

70) Seo SY, Kim YJ, Kim J, Nam MH, Park KY. Phytosphingosine induces systemic acquired resistance through activation of sphingosine kinase. Plant Direct.2021;5:e351.
71) Ennis M, Tiligada K. Histamine receptors and COVID-19. Inflamm Res. 2021;70:67-75.
72) Kumar SN, Mohandas C, Nambisan B.Purification of an antifungal compound, cyclo (l-Pro-d-Leu) for cereals produced by Bacillus cereus subsp. thuringiensis associated with entomopathogenic nematode. Microbiol Res. 2013;168:278–288.
73) Wertz PW. Lipids and the Permeability and Antimicrobial Barriers of the Skin. J Lipids.2018.
74) Bisht D, Arya RKK, Joshi T, Pal GR, Kumar R.(n.d.). REVIEW ON INDOLE DERIVATIVES USED AS ANTIULCER AGENTS.
75) Desai NC, Maheta AS, Jethawa AM, Pandit UP, Ahmad I, Patel H. Zeolite (Y-H)-based green synthesis, antimicrobial activity, and molecular docking studies of imidazole bearing oxydibenzene hybrid molecules. J Heterocycl Chem.2022;59:879–889.
76) Pandey P, Prasad K, Prakash A, Kumar V.Insights into the biased activity of dextromethorphan and haloperidol towards SARS-CoV-2 NSP6: in silico binding mechanistic analysis. J Mol Med.2020;98:1659–1673.
77) Du Y, Chen X. Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection. Clin Pharm Therap.2020;108: 242–247.
78) Yángüez E, Hunziker A, Dobay MP, Yildiz S, Schading S, Elshina E, … Dijkman R. Phosphoproteomic-based kinase profiling early in influenza virus infection identifies GRK2 as antiviral drug target. Nat Commun. 2018;9:1–13.
79) Kim SH, Han AR, Seo EK.Identification of Phytochemicals From the Caulis of Lonicera japonica. Chem Nat Compd.2016;52:918–919.
80) Gouvinhas I, Queiroz M, Rodrigues M, Barros AI. Evaluation of the Phytochemistry and biological activity of grape (Vitis vinifera L.) Stems: Toward a Sustainable Winery Industry. Polyphenols in Plants.2019;381–394.
81) Alagawany M, Farag MR, Abdelnour SA, Elnesr SS.A review on the beneficial effect of thymol on health and production of fish. Rev Aquac.2021;13:632–641.
82) Monajjemi M, Mollaamin F, Shojaei S. An overview on coronaviruses family from past to COVID-19: Introduce some inhibitors as antiviruses from Gillan's plants. Biointerface Res. Appl. Chem, (2020):10(3), 5575–5585.
83) Abaidullah M, Peng S, Song X, Zou Y, Li L, Jia R, Yin Z. Chlorogenic acid is a positive regulator of MDA5, TLR7 and NF-κB signaling pathways mediated antiviral responses against Gammacoronavirus infection. Int. Immunopharmacol., (2021); 96, 107671.
84) Villanueva S, Zhang W, Zecchinati F, Mottino A, Vore M. ABC transporters in extrahepatic tissues: pharmacological regulation in heart and intestine. Curr. Med. Chem., (2019); 26(7), 1155–1184.
85) Hu Z, Hu J, Ren F, Xu H, Tan MQW, B. R, Undefined. Nobiletin, a novel inhibitor, inhibits HBsAg production and hepatitis B virus replication. Elsevier. (2020).
86) Chen P, Nirula A, Heller B, Gottlieb RL, Boscia J, Morris J, Skovronsky DM. SARS-CoV-2 neutralising antibody LY-CoV555 in outpatients with Covid-19. NEJM., (2021); 384(3), 229-237.
87) Mokkapati M. The Role of Influenza Infection on Cell Metabolism, (2020).
88) Ikanovic T, Sehercehajic E, Saric B, Tomic N, Hadziselimovic R. In silico analysis of scopoletin interaction with potential SARS-CoV-2 target. In International Conference "New Technologies, Development and Applications" (2021, June);(pp. 897-903). Springer, Cham
89) Arora S, Itankar, P. Extraction, isolation and identification of flavonoid from Chenopodium album aerial parts. J Tradit Complement Med, (2018); 8(4), 476-482.
90) Ajaib M, Hussain Ashiq M, Shafi F, Hayat Bhatti K, Zahid MT. Antimicrobial and antioxidant analysis of Strobilanthes glutinous: an unexplored medicinal plant. Biosci. Res., (2020); 17(2):1521-1534
91) Schang Luis M. Discovery of the antiviral activities of pharmacologic cyclin-dependent kinase inhibitors: from basic to applied science, Expert Review of Anti-infective Therapy, (2005); 3:2, 145-149
92) Saadah LM, Deiab GIA, Al-Balas Q, Basheti IA. Carnosine to Combat Novel Coronavirus (nCoV): Molecular Docking and Modeling to Cocrystallised Host Angiotensin-Converting Enzyme 2 (ACE2) and Viral Spike Protein. Mol., (2020); 28;25(23):5605
93) Onocha PA, Ekundayo O, Eramo T, and Laakso I. Essential oil constituents of Chenopodium ambrosioides L. leaves from Nigeria. J Essent Oil Res. (1999);11, 220-222
94) Gupta D, Charles R, Mehta VK, Garg AN, Kumar S. Chemical examination of the essential oil of Chenopodium ambrosioides L from the southern hills of India. J Essent Oil Res, (2002); 14, 93-94
95) Tapondjou LA, Adler C, Boud AH, Fontem DA. Efficacy of powder and essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectants againstsix-stored product beetles. J Stored Products Res. (2002); 38, 395-402.
96) Parkash J, Kannubhai RP, Hepatoprotective activity of Chenopodium album leaves extract In Ccl4 induced hepatotoxicity in rats. Journal of Drug Delivery and Therapeutics, (2015); 5(2): 88-93.
97) Kaur M, Sharma S, Garg S, Arora M. Study of antibacterial activity of Chenopodium album leaves extract. Intl. J. Pharmacognosy and Phytochemical Res. (2018); 10(1): 1-4.
98) Külcü DB, Gökşk CD and Aydn S. An investigation of antibacterial and antioxidant activity of nettle (Urtica dioica L.), Mint (Mentha piperita), Thyme (Thyme serpyllum) and Chenopodium album L. plants from yaylac k plateau, giresun, Turkey". Turkish J. Agril. Food Scie. Technol. (2019); 7(1): 73 80.
99) Jesus RS, Mariana P, Robson BF, Thiele FB, Camilla FSA , Bianca VB, Natália Jank Mossmanna, Ritiel CC, Roberto CVS , Tanise VD, Bianca VB, Marli MAC, Liliane de FB. In vitro antimicrobial and antimycobacterial activity and PLC–DAD screening of phenolics from Chenopodium ambrosioides L. Braz. J. Microbiol., (2018); 49 (2): 296-302.Elsevier, PP153-169 https://doi.org/10.1016/B978-0-12-814849-5.00010-1