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Flavonoid Contents of Neotorularia torulosa and Its Antiviral Activity Against Coxsackievirus B4 In Vitro

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Abstract

Viral infections are the most catastrophic problem that affects living standards. This research aims to discover and evaluate new antiviral agents from plant sources. Antiviral activities of total alcoholic extract of *Neotorularia torulosa* were examined *in vitro* against Coxsackievirus B4 (CoxB4) using MTT assay. The MNTC (maximum nontoxic concentration) of plant extract was 62.5 µg /ml. The CC_{50}% (50% cytotoxic concentration) of *Neotorularia torulosa* plant extract different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 ug/mL**)** on Vero cells was found to be 192.01 ± 1.01 µg/mL while the IC_{50} (50% inhibitory concentration) against Coxsackievirus B4 (CoxB4) was 23.069% ± 0. 067 µg/mL. The antiviral activity of the plant extract reached 95.15 % at the maximum nontoxic concentration compared with 77.71 for Acyclovir drug indicating that the plant extract exhibit promising antiviral activity against CoxB4.

Seven flavonoid compounds were isolated from *Neotorularia torulosa* plant extract using various chromatographic techniques, including column chromatography, paper chromatography, and thin-layer chromatography (TLC). Final purification of flavonoid compounds was done using high-performance liquid chromatography (HPLC). Identification was carried out using different spectroscopic Analyses including UV and nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C-NMR).

1. Introduction

Natural products provide important medicines for treating various human and animal illness. They can be gotten from many biological resources including herbs. They represent an important source of high potency drugs to overcome the failure of traditional drugs [1,2].

Pathogenic microbes (viruses, fungi, bacteria, and parasites) responsible for infections and a variety of illnesses or disorders that have a direct impact on human health [3]. Due to increasing episodes of such resistance among pathogenic viruses encouraged the treatment with natural products. So, it's necessary to discover natural medicine that has low or zero side effects and a reliable cure for viruses [4,5].

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Coxsackievirus B4 (CoxB4) is a common human pathogen that causes some signs such as rash, fever and upper respiratory illness. This human enterovirus replicates at a high rate in the pancreas [6]. Severe pancreatitis lead to a broad range of diseases such as septic meningitis, myocarditis, hepatitis, gastroenteritis, pneumonia, and sudden death in neonates [7]. In the treatment of viral diseases, viral resistance to acyclovir has emerged as a problem especially in the treatment of immune compromised patients [8].

Natural Phytochemicals are beneficial in antiviral chemotherapy [9,10]. Available antiviral drugs are limited in their usefulness because they have little antiviral activities and exhibit differential toxicity towards patients. On the other hand, natural phytochemicals like Flavonoids can be used [11,12]. These drugs are active against a lot of illness producing viruses and effect on viruses through directing the viral toxicity at different phases. They have effect on viruses at different stages of their infection, like entrance of viruses, translation and replication of proteins. Viral infections lead to many diseases like AIDS, Herpes, Hepatitis, SARS, etc.
Viral diseases are widespread across the world. Curing different viral illness effectively is not obtainable. Vast number of flavonoids are therapeutically effective, most of them are investigated for their action against many viruses.

Flavonoid compounds have minimum toxicity activity, strong inhibition effect and no detection for cell proliferation at the examined doses. Most of them effectively prevented viral infection by protecting the mice examined prophylactically from deadly dosage of virus (used in the *in vivo* studies). Some glycosides of flavonoid have high solubility than that of the free form of that flavonoid and this lead to increase antiviral activity of them. Many flavonoids had stronger antiviral effect than the corresponding drugs which used [13].

Neotorularia troulosa belongs to the family Cruciferae (Brassicaceae) which is one of the largest families in the plant kingdom that is rich in medicinal plants. Most of the *Brassicaceae* species are important vegetables consumed worldwide due to their particular essence, aroma, and flavor, but mainly for their broadly recognized functional properties [14]. These properties are directly related with their phytochemical composition of high biological value in the treatment of diseases, owing to their bioactive properties like anti-obesity, anticancer, antimicrobial, antioxidant, hepatoprotective, cardioprotective, gastroprotective, anti-inflammatory, antiemetic, and immunomodulatory [15].

Neotorularia troulosa grows at Sinai proper. Phytochemical screening was carried out on *Neotorularia troulosa* which showed the presence of several chemical compounds, including alkaloids, flavonoids, Saponin, tannins and phenolic. This study also showed that the alcoholic extract of the plant has high antifungal activity against *Candida albicans* [16].

In this work, antiviral activity the alcoholic extract of *Neotorularia torulosa* was evaluated. In addition to isolation of some flavonoid compounds from the plant and identified

2. Experimental

2.1. Material of plant

The over ground parts of *Neotorularia torulosa* were gathered during spring during the year 2023 from Sinai Middle, Egypt, and identified by Desert Research Centre and a comparison to the Flora of Egypt's plant descriptions [17,18]. The collected plant materials were dried in air and in shadow then ground into a fine powder.

2.2. Phytochemical studies

Plant Extraction

One kilogram of powdered of *Neotorularia torulosa* was extracted using ethanol (80%) by maceration method, the ethanolic extract was filtered, this process was repeated till exhaustion. The prepared extract was concentrated using Rotavapor at temperature below 40 °C under low pressure till complete dryness then weighed 100 g of crude extract. The extract was dissolved in small volume of hot water, filtered to remove lipids and chlorophyll, and then concentrated to dryness and redissolved in small volume of absolute methanol with continuous stirring to get tired of salts, re-filtered, and evaporated to dryness, giving 80 g of purified extract.

Chromatographic investigation

Approximately 0.1 ml of alcoholic extract subjected to paper chromatography (PC, Whatman No.1) and thin-layer chromatography (TLC) using different systems:
Systems for (PC).
a. BAW (Butanol- acetic acid- water) (4/1/ 5 v/v/v) clear phase.
b. 15 % Ac OH (Ac OH - water) (15/85 v/v).
Systems used in TLC.
c. EaOAc- CH_3OH- H_2O (30/5/4 v/v/v)).
d. EaOAc- CH_3OH- H_2O (70/ 5/ 4).
e. $CHCl_3$- CH_3OH (95/ 5).

Investigation of the spots was done under UV lamps, with and without exposing to ammonia vapors and then Spraying with $ALCL_3$ solution. Examination showed that extract containing many spots characteristic of flavonoids**.**

Purification of flavonoids

Flavonoid compounds were isolated from the extract using a polyamide column (1.5 × 2 cm).

The extract was dissolved in a minimal volume of water, then applied over polyamide column packed with water. Elution started with water, followed by gradual increase with ethanol.

Collected fractions were concentrated under vacuum and subjected to paper chromatography using developing systems (a) and (b). Similar fractions were collected then applied to Whatman No.3 preparative paper chromatography using developing system (b), visualized the bands under UV light

and eluted with 70% methanol. The eluates were dried and then purified using Sephadex column (LH-20) elution was done by methanol then gradual increase with water.

The sub-fractions collected from Sephadex column were tested for their purity by subjection to TLC by using system c. Final purification of flavonoid compound, the High-Performance Liquid Chromatography (Agilent 1200 series) provided with DAD (Diode Array Detector) and column C_{18} RP) was used, the system used is H_2O gradually increased by acetonitrile.

Pure flavonoid compounds were identified using R_f, and physical methods; spectrophotometer and Nuclear Magnetic Resonance (400 MHz).

Antiviral effects

Coxsackievirus B4 virus was obtained from Virology Laboratory, Faculty of Medicine, Al-Azhar University, and Science Way laboratory for Scientific Researches and Consultations, Egypt. DMEM media was purchase from Serana company, DMSO was purchased from molekula company.

Determination of cytotoxicity of plant extract on Vero cell

Maximum non-toxic concentration for the *Neotorularia torulosa* plant extract on vero cells was measured by MTT colorimetric method. Different concentrations of tested samples were prepared. Once a confluent monolayer of Vero cells was formed in 96-well micro titer plates (take about 24 hours), the growth medium was decanted, and the cell monolayer was washed twice with wash medium. Double-fold serial dilutions of the test sample were prepared using minimum essential media (DMEM). A volume of 0.1 ml from each dilution was added to separate wells, while three wells were reserved as controls and received only maintenance medium. Plates incubation at 37 °C and observed periodically over a 48-hour. Cells were examined for signs of cytotoxicity, such as complete or partial detachment of the monolayer, cell granulation, shrinkage or rounding. We prepared MTT solution (5mg/ml in PBS) (BIO BASIC CANADA INC). A volume of 20 µl MTT solution was added to each well, followed by putting on a shaking platform at 150 rpm for 5 minutes to ensure proper mixing of MTT into the. The plates were then incubated at 37 °C in a 5% CO_2 atmosphere for 4 hours to allow metabolic conversion of MTT. Then the media was Dumped off by using paper rubs to dry plate to remove residue. In 200ul DMSO Formazan was suspended (metabolic product of MTT). To let formazan mixed into the solvent; shaking on platform for 5 minutes at 150 rpm was done.

The optical density was measured at 560 nm, and background absorbance was removed at 620 nm. The count of cells and optical density must be directly proportional.
MNTC for plant extract was measured then can be used for more biological [19,20].

2.3 Assay for Antivirus

Protocol for MTT Analysis

A total of 10,000 cells were seeded in 200 µl of culture medium per well in a 96-well plate. For blank controls three wells were left empty. To let the cells joined to the wells; incubate overnight at (37°C, 5% CO_2). Incubate nonlethal dilution from tested sample (different concentrations 62.5, 31.25, 15.62 and 7.81 ug/mL) with suspension of the virus in same volume (1/1)) for 1 hour. A volume of 100 µl from the sample or viral suspension were added and shacked at 150rpm for 5 minutes.

Incubated sample /Viral suspension at (37C, CO_2 (5%)) for one day to obtain the virus affect. Add 20 uL MTT solutions to each well. For 5 minutes put it over a shaking table 150 rpm to let MTT mix with media. Plates were incubated at 37 °C with 5% CO_2 for 1–5 hours to permit metabolic conversion of MTT prior to medium removal. The medium was carefully discarded, and the plate was gently dried to remove any remaining residue. The formazan crystals, produced by MTT metabolism, were dissolved in 200 µl of DMSO. To ensure complete dissolution of formazan in the solvent, the plate was placed on a shaking platform at 150 rpm for 5 minutes. The optical density was measured at 560 nm, and background absorbance was removed at 620 nm. The count of cells and optical density must be directly proportional [21].
To calculate the antiviral activity, the following equation was used:

$$\textbf{Antiviral activity}\ (\%) = \frac{\textbf{optical density of treated cells} - \textbf{optical density of virus control}}{\textbf{optical density of cell control} - \textbf{optical density of virus control}} \times 100$$

3. Results

Separation and identification of flavonoids

Identification of isolated compounds from *Neotorularia torulosa* was done by physical methods including UV and NMR spectral data (^{1}H and ^{13}C-NMR; one- and two-dimensional)

which were measured at 400.1 and 150.91 MHz, respectively. Two-dimensional proton- carbon correlations were done by using HMBC (hetero nuclear multiple- bond correlation) pulse sequences and compared with previously published data [22-28].

The isolated compounds (H1-H7) were identified as follow:

Compound H1

Kaempferol: yellow crystals, R_f (0.91) using system (c) UV: λmax (MeOH): (nm) 267,367, (NaOMe) 275,416, ($AlCl_3$) 268,350,424, ($AlCl_3$/ HCl) 269,348,424, (NaOAc) 27,303,387, (NaOAc/H_3BO_3) 36,302,372.

^{1}HNMR

δ 8.1 (dd, 2H, J (8 , 2.5 Hz) for H6' ,H2'), δ 6.81 (d, 2H, J(8.0 Hz) for H5'and H3'), 6.62 (d, 1H, J (8.50 Hz for H8), 6.35(d ,1H, J(2.5Hz)for H6).

Compound H2

kaempferol -3-O-β-D-glucoside: crystals yellow in color, R_f (0.41) using system (c). Ultraviolet: λmax in CH_3OH (nm) 266, 251, 353, ($NaOCH_3$) 262,329,390, ($AlCl_3$) 274,342,411, ($AlCl_3$/HCl) 27,342,411, (NaOAc) 269,342,382, (NaOAc/H_3BO_3) 260, 375.

^{1}HNMR, (DMSO –d_6) 8.0 (d, 2H, J (8.50 Hz) for H2' and 6'), 6.8 (d, 2H, J (8.50 Hz), H5' and 3'), 5.61 (d, 1H, J(2.50 Hz), H6) and 5.55 (d, 1H,J(2.50 Hz), H8), 5.0 (d, J(7.0 Hz), 1H, H1" anomeric glucose sugar) and 3.2-3.6 (m. remaining glucose protons).

^{13}C NMR: ppm 174.5 (C4), 160. 5 (C7), 159.67 (C5), 157.55 (C 4'), 153.21 and 153.22(C2 and C9 respectively), 132.6 (C3), 130. 0 (C2',6'), 121.0 (C1'), 115 (C3',5'), 103.0 (C10), 98.67 and 95.4 (C6 and C8 respectively), 102.2 (C1″ glucose), 79. (C3″and C5″), 75.4 (C2″), 71.2 (C4″). 60.1 (C6″)

Two - dimensional carbon – proton correlation spectrum of compound H2 show that

1-Correlation between δ 5.0 (H1"for glucose) and 132.6 (C3 for kaempferol)

2-Correlation between 8 (H2' and H6' for kaempferol) , 130 (C2' and C6' for kaempferol)

3-Correlation between δ 6.8 (H-3' and 5' for kaempferol) and 115 (C3'and 5' for kaempferol).

Compound H3

Kaempferol-7-O-glucoside: crystals yellow in color, R_f (0.36) using system (c). UV λmax (MeOH) (nm) 262, 367, (NaOMe) 273,295,440, ($AlCl_3$) 266,353,424, ($AlCl_3$/ HCl) 265,422, (NaOAc) 262,366,420, (NaOAc/H_3BO_3) 262.366.420.

^{1}HNMR (DMSO–d_6) δ 7.9 (d, 2H, J (8 Hz) H2' and H6'), 7 (d, 2H, J(8 Hz), H5' and H3'), 6.3 (d, J(2.5Hz), 1H, H8), 6.14 (d, 1H, J(2.50 Hz), H6), 5.3 (1H,d, J(7 Hz), H1" anomeric glucose proton) 3.3- 3.8 (remaining protons of glucose).

^{13}C NMR: ppm 177.3 (C4), 165.1 (C7), 161.2 (C5), 156.6 (C4'),160.2 and 160.9(C2 and C9 respectively), 133.6 (C3), 131.25 and 131.14 (C2'and C6'), 120 (C1'), 115.9 (C3' and C5'), 103.45 (C10), 98.65 and 95.55 (C6 and C8 respectively), 101.7 (C-1″ glucose), 78.0 (C3″and C5″), 76.5(C2″), 70.25 (C4″). 61.0 (C6″).

Compound H4

Kaempferol-3, 7- O-β-D diglucoside: yellow crystals, R_f =0.57 using system (c). UV: λmax (MeOH) (nm) 268.347, (NaOMe) 268,380, ($AlCl_3$) 276,355,400, ($AlCl_3$/ HCl) 276,352,400, (NaOAc) 268, 346, (NaOAc/H_3BO_3) 268,346.

^{1}HNMR (DMSO–d_6) δ 8 (d, J (8 Hz), 2H, H2' and 6'), 7.4 (d, J (8.0 Hz), 2H, H5'and 3'), 6.7(d, J (2.5Hz), 1H, H-8), 6.14 (d, J (2.5 Hz), 1H, H6), 5.2 (d, 1H, J (7 Hz), H-1" anomeric glucose proton), 5 (d, J (7 Hz), H-1'" anomeric glucose proton) and 3.2- 4 (remaining sugar protons).

Compound H5

Quercetin: crystals, yellow in color, R_f (0.82) using system (c). Ultraviolet: λmax (MeOH) (nm) 250, 300,.371, (NaOMe) 276,425, ($AlCl_3$) 263,386, ($AlCl_3$/ HCl) 273,355,425, (NaOAc) 273,326,389, (NaOAc/H_3BO_3) 263,386.

^{1}HNMR: 7.73 (d, J (8.5 Hz), 1H, H2'), 7.5 (dd, J(8.5,2.5 Hz), 1H, H6'), 6.85(d, J(8.5 Hz), H5'), 6.5 (d, J(2.5) , H6) and 6.21 (d, J(2.5 Hz), H8).

Compound H6

Quercetin-3-glucoside-4` methyl ether: crystals yellow in color, R_f (0.77) using system (c). Ultaviolet: λmax (nm) (MeOH): 260,358, (NaOMe) 270,430, ($AlCl_3$) 270,440, ($AlCl_3$/ HCl) 270,440, (NaOAc)276,395, (NaOAc/H_3BO_3) 276,395.

^{1}HNMR (DMSO–d_6) δ 8 (doublet,1H, J (8.5 Hz), H2'), 7.8 (Q, J (8.5 ,2.5 Hz), H6'), 6.85 (doublet, J (8.5 Hz), H5'), 6.5 (doublet, J (2.5Hz), H6), 6.21 (doublet, J (2.5 Hz), H8), 5.4 (1H, doublet, J(7 Hz), H-1" anomeric glucose proton), 3.9 (S,4`-OCH_3) and 3.1-3.9 (sugar proton).

^{13}C NMR (Methanol-D_6): ppm 177.8 (C4), 165. (C7), 162.0 (C5), 157.0 (C2), 156.5(C9), 150 (C4'), 146 (C3'), 135 (C3), 122 (C1'), 121 (C6'), 116 (C2'), 112.1 (C5'), 105.0 (C10), 98.3 and 94 (C6 and C8 respectively), 102.0 (C1″), 75.49 (C5″), 77.0 (C3″), 73.18(C2″), 71.34(C4″),62.74 (C-6″) and 65.1(OCH_3).

Compound H7

Quercetin-3-glucoside-4`,7 dimethyl ether: crystals yellow in color, R_f =0.82 using system (c). UV: λ max (nm), (MeOH): 257,352, (NaOMe) 258,330,400, ($AlCl_3$) 275,355,405, ($AlCl_3$/ HCl) 275,355, 405, (NaOAc) 258,360, (NaOAc/H_3BO_3) 259,360.

^{1}HNMR (DMSO–d_6) 8.2 (doublet,1H, J (2.5 Hz). H2'), 8.0 (Q, J (8.5 2.5 Hz), H6'), 7.0 (doublet,1H, J (8.5 Hz), H5'), 6.5 (doublet,1H, J (2.5Hz), H8), 6.3 (doublet,1H, J (2.5 Hz), H6), 5.4 (doublet, J (7 Hz), H-1" anomeric glucose proton), 3.86 (S,4`-OMe), 3.84 (S,7-OCH_3), and 3.2-4 (sugar protons).

^{13}C NMR (Methanol-D_6): ppm 178.32 (C4), 166 (C-7), 161.55 (C5), 157 (C2), 156.86 (C9), 150.77 (C4'), 147 (C3'), 134.64 (C3), 123.13 (C1'), 123 (C6'), 116.25 (C2'), 112 (C5'), 105.63 (C10), 98.60 and 92.86 (C-6 and C-8 respectively), 102.21 (C1″), 77 (C3″), 74 (C5″), 72(C2″), 69 (C4″),60.84 (C6″),56.78 (4'-OCH_3) and 56.27(7-OCH_3).

HMBC of compound H7 in DMSO showed (Hetero nuclear multiple bond correlation

Two-dimensional carbon – proton correlation spectrum of compound H7 show that

1- Correlation between δ 3.86 (4`-$OCH_3$) and 150.77 (C-4` for Quercetin)

2-Correlation between δ 3.84 (7-OCH_3 for Quercetin) and 166 (C-7 for Quercetin).

3-Correlation between δ 5.4 (H-1" anomeric proton of

glucose) and 134.64 (C-3 for Quercetin)
Structure of isolated compounds are shown in Fig (1).

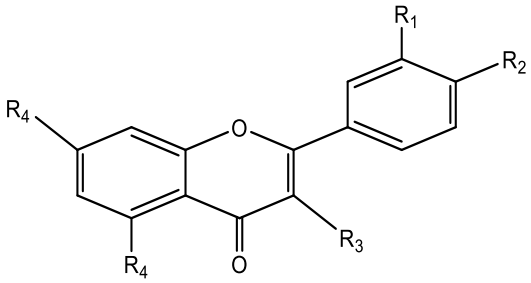

Fig. 1. Structure of isolated compounds from Neotorularia torulosa.

Compound	R_1	R_2	R_3	R_4	R_5
H1	H	OH	OH	OH	OH
H2	H	OH	O-glucose	OH	OH
H3	H	OH	OH	OH	O-glucose
H4	H	OH	O-glucose	OH	O-glucose
H5	OH	OH	OH	OH	OH
H6	OH	OCH_3	O-glucose	OH	OH
H7	OH	OCH_3	O-glucose	OH	OCH_3

Antiviral Activity

The antiviral potential of the tested plant extract on CoxB4 virus was investigated using the MTT antiviral assay. AS shown in table (1) The CC_{50} value is 192.01 ± 1.01 µg/ mL.

CC_{50} is identified as the concentration of the plant extract required to decrease 50% of cell viability. (The concentration caused 50% toxicity).

The IC_{50} (inhibitory concentration of 50% value) is 23.069 ± 0.067 µg/mL compared to Acyclovir 360.92 ± 0.011 µg/mL as shown in table (3).

According to tables (1- 3) the plant extract show high activity against CoxB4 virus with MNTC for extract of 62.5 µg/mL and antiviral activity for MNTC is 95.15 compared with Acyclovir MNTC 62.5 µg/mL and antiviral activity 77.71%.

Results indicated that the extract exhibited significant antiviral activity against Coxsackievirus B4 (CoxB4) with non-toxic concentration. This antiviral activity is likely attributed to the high flavonoid content present in the plant extract. Previous study showed that some of these isolated flavonoids have strong antiviral activities close to available commercial drugs [5].

Microscopic images illustrate the effects of different concentrations of *Neotorularia torulosa* extract on replication of Cox B4 virus are shown in figure 2.

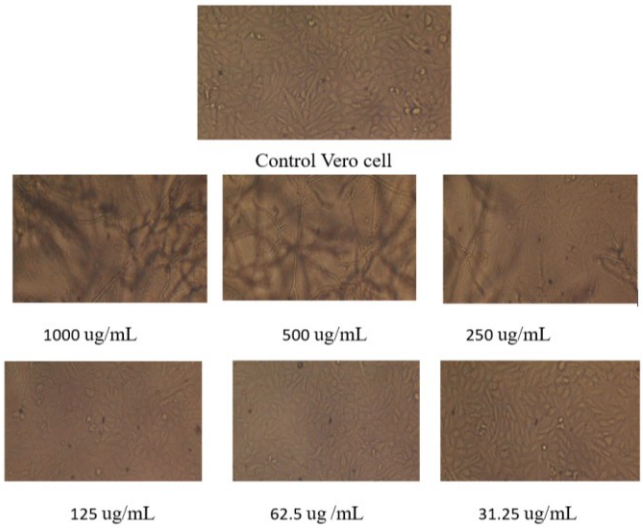

Fig (2). Microscopic images illustrating the effects of different concentrations of plant *Neotorularia torulosa* extract on replication of Cox B4 virus.

4. Discussion

Antiviral activities of total alcoholic extract of *Neotorularia torulosa* were examined *in vitro* against Coxsackievirus B4 (CoxB4) using MTT assay. The MNTC of plant extract was 62.5 µg /ml which means that the plant is safe when used. The CC_{50}% (50% cytotoxic concentration) of *Neotorularia torulosa* plant extract different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 ug/mL**)** on Vero cells was found to be 192.01 ± 1.01 µg/mL while the IC_{50} (50% inhibitory concentration) against Coxsackievirus B4 (CoxB4) was 23.069% ± 0. 067 µg/mL. The antiviral activity of the plant extract reached 95.15 % at the maximum nontoxic concentration compared with 77.71 for Acyclovir drug indicating that the plant extract exhibit promising antiviral activity against CoxB4. This activity is due to high content of flavonoids in plant, [5]. Previous studies indicate that flavonoid compounds isolated from the plant extract have potent antiviral activities; like Kaempferol, quercetin and their derivatives [13,29-37].

Conclusion

This current study focused on antiviral activity of Neotorularia torulosa at nontoxic concentrations against Coxsackievirus B4 in vitro which demonstrated significant inhibitory activities against it.

This finding highlights the potential of Neotorularia torulosa to complement the current therapeutic resources against viral infections, offering a promising foundation for future research into the development of antiviral therapies.

Table (1): The cytotoxic concentration 50% (CC_{50}) of *Neotorularia torulosa* extract

ID	µg/mL	Optical density			Mean Optical density	±SE	Viab. %	Tox. %	CC_{50}± SD
Vero		0.722	0.717	0.715	0.718	0.00208	100	0	µg/mL
Extract	1000	0.03	0.03	0.03	0.030	0	4.178273	95.821	192.01 ± 1.01
	500	0.03	0.03	0.03	0.030	0	4.178272	95.821	
	250	0.185	0.196	0.194	0.192	0.00338	26.69452	73.305	
	125	0.57	0.553	0.561	0.561	0.00491	78.18013	21.819	
	62.5	0.715	0.711	0.717	0.714	0.00176	99.48932	0.5106	
	31.25	0.717	0.721	0.716	0.718	0.00153	100	0	

SE: Standard Error; SD: Standard Deviation; Viab.: Viability; Tox.: Toxicity; CC_{50}: 50% cytotoxic

Table (2): The antiviral activity of *Neotorularia torulosa* extract against CoxB4 virus

Test	uM	Optical density			Mean Optical Density	S E	Viab%	Tox. %	%Activity of Virus	% of Anti-viral activity
Vero Cont.		0.721	0.714	0.719	0.718	0.001	100	0		
CoxB4		0.309	0.316	0.311	0.312	0.001	43.45	56.545	100	0
Ext. conc.	62.5	0.70	0.692	0.703	0.698	0.002	97.26	2.739	4.844	95.15
	31.25	0.588	0.605	0.598	0.597	0.004	83.14	16.852	29.802	70.19
	15.62	0.43	0.444	0.451	0.441	0.005	61.51	38.486	68.062	31.93
	7.81	0.318	0.31	0.314	0.314	0.002	43.73	56.267	99.507	0.492

SE: Standard Error; Viab.: Viability; Tox.: Toxicity.

Table (3): Cytotoxicity, MNTC (µg/mL) and antiviral activity of Neotorularia torulosa extract against CoxB4 virus

Sample	MNTC	IC_{50}	Antiviral activity
Plant extract	62.5	23.6 ± 0.67	95.15
Acyclovir	62.5	360.92 ± 0.011	77.71

MNTC: Maximum Non-Toxic Concentration, IC50: inhibitory concentration of 50%

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