IJBPAS, February, 2022, 11(2): 715-731

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STRUCTURAL CHARACTERIZATION AND BIOACTIVITY OF THE PHYTOCOMPOUND ISOLATED FROM STEM EXTRACT OF *Euphorbia milii* Des. Moul.

A.CH. PRADYUTHA[1*] AND V. UMAMAHESWARA RAO[2]

1: Dept. of Microbiology, R.B.V.R.R Women's College, Narayanguda, Hyderabad, Telangana State

2: Dept. of Botany and Microbiology, Acharya Nagarjuna University, Nagarjunanagar- 522510, Guntur District, A.P

***Corresponding Author: A.Ch.Pradyutha: E Mail: pradyutha.g@gmail.com**

Received 15th March 2021; Revised 16th April 2021; Accepted 1st June 2021; Available online 1st Feb. 2022

https://doi.org/10.31032/IJBPAS/2022/11.2.5890

ABSTRACT

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources particularly of plants, based on their use in traditional medicine. *Euphorbia milii* plant has been used in folk medicine in some countries like China, Nepal to treat some diseases. The present investigation was aimed to isolate and characterize the bioactive compound from methanol extract of *E. milii* stem which was found to be pharmacologically potent when compared with other extracts. This extract of the stem was separated into individual fractions by column chromatography and the fraction that exhibited very high antimicrobial activity against tested bacteria and fungi was elucidated to obtain the pure compound and characterized the structure. The isolated active compound was subjected to ^{1}H NMR, ^{13}C NMR, and Mass spectral analyses. From the spectral data obtained, the compound was identified as Quercetin-flavonoid. The isolated quercetin compound exhibited high antimicrobial activity against tested bacterial and fungal pathogens and also owned good cytotoxic activity. This research work has successfully led to the characterization of bioactive compound from the stem extract of *E. milii*.

Keywords: *Euphorbia milii*, Column Chromatography, ^{1}H NMR, ^{13}C NMR, Quercetin, Flavonoid

INTRODUCTION

Plants were the major source of traditional medicine. Plant-derived medicine is widely used in traditional cultures all over the world. Plants synthesize various complex organic chemicals called phytochemical compounds, able to fight and attack other plant species in their proximity by suppressing their growth **[1]**. Certain phytochemical constituents perform a crucial role in human health also **[2]**. Plant parts like stem, flowers, and roots possess so many phytoconstituents, that exhibit biological potential on several disease conditions **[3]**. The role of plants in human health is significant, especially in contemporary medicine where the presence of natural products is increasing at a rapid rate as an alternative to synthetic chemicals. WHO (World Health organization) stated that 80% of the emerging world's community depends on conventional medicine for treatment **[4]**.

The family *Euphorbiaceae*, generally named as Spurge family, contains mostly monoecious herbs, shrubs, and trees, some of which are cactus-like and succulent. Euphorbiaceae is the most abundant family of plant life and the plants are characterized by the occurrence of milky sap **[5]**. One of the traditionally used medicinal plants of the Euphorbia species, which has potential medicinal value is *Euphorbia milii* Des Moul., generally called "Christ plant". It is an ornate plant innate to Phillippines and Madagascar, but also broadly spread in India. *E. milii* is extensively utilised in folk medicine for the remedy of warts in South Brazil, for hepatitis and cancer in China **[6]**. Visioning the importance of natural bioactive products towards new drug principles as well as the medicinal treasure of plants, the present work was focused to isolate and characterize the bioactive compound from the stem extract of *Euphorbia milii* plant and its screening for bioactivity.

MATERIALS AND METHODS

Microorganisms

To check the antimicrobial potential of the isolated pure compound, nine Gram-positive, six Gram-negative bacteria and three plant pathogenic fungi, collected from Microbial type culture collection and Gene Bank, Chandigarh, India.

***Gram-Positive bacteria*:**

Arthrobacter protophormiae MTCC 2682, *Micrococcus luteus* MTCC 106, *Bacillus subtilis* MTCC 441, *Rhodococcus rhodochrous* MTCC 265, *Bacillus megaterium* MTCC 428, *Staphylococcus aureus* MTCC 737, *Enterococcus faecalis* MTCC 439, *Streptococcus mutans* MTCC

497, *Lactobacillus acidophilus* MTCC 10307and *Staphylococcus aureus* MTCC 737.

***Gram-Negative bacteria*:**

Salmonella enterica MTCC 3858, *Alcaligens faecalis* MTCC 126, *Proteus vulgaris* MTCC 426, *Pseudomonas aeruginosa* MTCC 1688, *Proteus mirabilis* MTCC 425 and *Enterobacter aerogenes* MTCC 10208.

***Fungi*:**

Fusarium oxysporum MTCC 7392, *Sclerotium hydrophilum* MTCC 2157 and *Alternaria sps*. MTCC 9692.

***Cancer Cell Lines*:**

Human Cervical cancer cell line (HeLa), Human alveolar adenocarcinoma cell line (A549), Human breast adenocarcinoma cell line (MCF-7), Human chronic myelogenous leukemia cell line (K562) and Human embryonic kidney cell lines (HEK 293) used in this study were procured from NCCS (National Center for Cell Science), Pune, India.

Purification of compounds from potential extracts by Chromatographic studies:

***Thin-layer chromatography (TLC)*:**

Thin layer chromatography (TLC) is especially helpful for checking the purity of fractions **[7]**. Traditional one-dimensional ascending method was adopted to screen the purity of each solvent extract. Silica gel 60F254 (Merck) TLC plates of 7X6 cm size were used for this process. The sample was applied on the plate at about 1.5 cm from the bottom edge and plate markings were made. To spot the sample on the TLC plates, glass capillaries were used. TLC plate was then put in a container holding a proper solvent system for development through capillary action. After saturation with the mobile phase for 20 minutes, the plate was taken out of the developing chamber and allowed to dry completely. The developed chromatogram was analyzed by short-wavelength (254 nm) ultraviolet light.

Column-chromatography is an effective technique practiced in the separation of crude plant extract components into their pure form **[8]**. The methanolic extract of the stem that exhibited potential antimicrobial activity was separated into different fractions by column chromatography with silica gel **[9]**. The silica gel of 100-200 mesh size was used for column packing by the slurry technique. The slurry made with hexane and packed up to 75 cm length of a glass column having the dimensions of 100 cm length and 2 cm diameter. The top of the column was covered with sterile non-absorbent cotton to stop disturbances of the surface during consequent loading. The sample to be purified was dissolved in a small amount of methanol and

flowed in a funnel to the packed column. The solvent level controlled at 2.5 cm above the extract. A solvent system of chloroform - ethyl acetate and ethyl acetate - methanol in different fractions was used to run the column and elutes were collected for the isolation of active components from stem extract of *E. milii.*

Characterization of purified fraction:

The isolated compound was identified using spectroscopic techniques **[10, 11]** namely ^{1}H NMR (500 MHz) and ^{13}C NMR (100 MHz), and Mass spectrometry (ESI-TOF-MS).

^{1}H NMR and ^{13}C NMR

To elucidate the structure of secondary metabolites from natural products, ^{1}H NMR and ^{13}C NMR spectroscopic techniques are significant as carbon and hydrogen are the richest atoms of natural products **[12]**.

^{1}H NMR spectra of the isolated compound were read at 500 MHz with a Bruker Avance-400 spectrometer. Data was recorded as position (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, quint= quintet, m = multiplet), coupling constant (J in Hz) and integration (number of protons). Residual methanol (δ = 7.26 ppm) was used as an internal reference for ^{1}H NMR spectra. ^{13}C NMR spectrum was recorded at 100 MHz using Bruker Avance-400 spectrometer. Residual methanol (δ = 77.1 ppm) was used as an internal reference for the ^{13}C NMR spectrum.

***Mass Spectrometry*:**

The mass spectrometry technique is useful to detect the exact molecular formula of the compound based on the positions at which a molecule was fragmented **[13]**. The mass spectrometer was operated in positive electrospray ionization mode and the spectrum was noted through scanning the mass scale m/z 50–500. Nitrogen used as drying, nebulising and collision gas at a flow rate of 12 L/min. The capillary heat was maintained at 350°C and nebulizer pressure at 45 psi.

Antimicrobial screening of the purified compound:

The biological action of the isolated compound was tested on bacteria and fungi by using the agar well diffusion technique **[14]** and the inhibition zone was measured. Bacterial and fungal suspensions were made individually and 0.3 ml of each bacterial and fungal suspension was added in separate 15 ml aliquots of sterilized melted state nutrient and potato dextrose agar medium, individually and poured into sterilized Petri dishes. Wells were bored on the surface of agar plates with a cleaned cork borer of 6mm in diameter after solidification of the medium. A little amount of agar suspension

was poured into the wells, before adding 100µl of the sample prepared by suspending 100mg of sample in 1ml of dimethyl sulfoxide. The plates were incubated at 37˚ C for 24 hrs. After 24hrs of incubation, the zone of inhibition was measured. For individual sample and bacterial species, triplicates were prepared. Streptomycin (for bacterial species) and fluconazole (for fungal species) were used as positive controls with 10 µg/ml concentration.

Determination of MIC, MBC and MFC:

Minimum inhibitory concentration (MIC) was screened by using a broth dilution technique **[15]** at different concentrations viz.,1.56mg/ml, 3.12mg/ml, 6.25mg/ml, 12.5mg/ml and 25 mg/ml. Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) were assessed on those strains of bacteria and fungi, which exhibited a zone of inhibition against the pure compound isolated from the stem extract of *E. milii*.

Anticancer activity:

The in vitro anticancer activity of the purified compound was estimated using MTT (3-(4, 5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) staining process **[16]**. Individual cells were incubated at a density of 5 × 103 cells well-1 in 96-well plates for 12 h. Further, the cells were managed with different concentrations of test compound and incubated at 37°C for 48 hrs. Then, 20µl of MTT solution (5 mg ml−1 in PBS) was added to all wells and again incubated for an additional period of 4h at 37°C. The purple coloured pharmacological crystals produced were dissolved in 150µl well−1 of DMSO and the cell proliferation (%) was noted at 570 nm within a microplate reader (BioRad Laboratories, Hercules, CA, USA) and the results were compared with doxorubicin, the positive control. The results were represented as the mean values of triplicate experiments.

The IC50 values (50% inhibitory concentration) were determined from the log concentration-effect curves in Graph Pad Prism (Graph Pad software Inc., CA, USA) using non-linear regression analysis, for all cell lines used and for the tested drug. Growth inhibition percentage of cells treated with compounds was calculated by using the following formula

% Inhibition = 100 − (Test OD/Non-treated OD) x 100

RESULTS AND DISCUSSION

The methanol extract of the stem was subjected to column chromatography with silica gel using chloroform and ethyl acetate (9:1) as well as ethyl acetate and methanol (9:1) as solvent systems. Totally, 38 fractions were obtained during column

chromatography procedure. The fractions with similar Rf values and spotting patterns on the TLC plate were pooled and thereby finally achieved 24 fractions. During the antimicrobial screening of all the fractions, SM-13 and SM-16 fractions exhibited the antimicrobial activity. The fraction SM-13 exhibited high antimicrobial activity and showed purity in the TLC plate. The pure fraction compound with high antimicrobial activity was analyzed using spectroscopic techniques for elucidation of the chemical structure.

Characterization of pure compound:

The pure compound obtained from the methanol extract of the stem of *E. milii* was a pale yellow colour substance.

^{1}H NMR spectrum of the purified compound exhibited the following signals **(Figure 1)**.

^{1}H NMR spectra (500 MHz, CD3OD-d6) δ: 6.80 (2H, d, H-6), 6.54 (1H, d, H-8), 7.07 (1H, d, H-2′), 6.79 (1H, d H-5′), 6.85 (1H, d, H-6′). The 1H NMR spectrum of the pure compound showed the signals of aromatic protons, which are similar to those reported for Quercetin moiety.

The ^{13}C NMR spectra of the purified compound showed the following signals **(Figure 2)**.

^{13}C NMR (100 MHz, CD3OD-d6) δ: 97.65 (CH, C-8), 102.15 (CH, C-6), 104.7 (C, C-10), 116.63 (CH, C-2', C-5'), 119.81 (CH, C-6'), 126.05 (C, C-1'), 132.93 (C, C-3), 146.45 (C, C-3').

The mass spectrum peak at 302 communicates to M+ ion of the isolated pure compound gave the significant fragmentation peaks at [M+H-H2 O]+ =285. [M+H-H2 O-CO]+ =257. [M+HH2 O-2CO]+ =228. [M+H-CO]+ =273. [M+H-2CO]+ =245 **(Figure 3)**. These peaks corresponds to $C_{15}H_{10}O_7$ which confirm the mass of quercetin.

From the above ^{1}H NMR, ^{13}C NMR and Mass spectral data, the compound was confirmed as Quercetin with the structure given in **Figure 4**. Quercetin (3,3',4',5,7-pentahydroxyflavone) belongs to the class called flavonols that cannot be synthesized by the human body **[17]**. Identification and characterization of the bioactive compound, quercetin from *E. milii* stem extract in this present study is the first report. Previously, Kamurthy *et al*. **[18]** have stated the presence of quercetin in the flowers of *E. milii*. A few research publications have declared that the production of secondary plant metabolites is mainly based on environmental conditions and these are affected at the gene level and the genetic diversity of plants **[19]**. Therefore, these could be a few reasons for explaining the difference between the earlier

and present reported results of the isolated compounds.

Antimicrobial activity of Quercetin:

Antibacterial Activity: The quercetin compound isolated from the methanol extract of the *E. milii* stem confirmed antibacterial potency towards both Gram-positive and Gram-negative bacteria **(Table 1 & Plate 1)**. *Rhodococcus rhodochrous* and *Enterobacter aerogenes* exhibited resistance against quercetin. The highest zone of inhibition observed with *Pseudomonas aeruginosa* was 16.2 mm. Of the Gram-positive and Gram-negative bacteria tested, *Arthrobacter protophormiae*, *Bacillus megaterium*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Alcaligens faecalis*, *Proteus vulgaris*, *Salmonella enterica*, *Pseudomonas aeruginosa* exhibited higher sensitivity to quercetin than that of standard antibiotic streptomycin. These results are indicating the broad-spectrum activity of the quercetin.

Antifungal activity:

The isolated pure compound, quercetin showed antifungal activity against the three fungal pathogens tested **(Table 2)** with some variation among the species. *Fusarium oxysporum* and *Alternaria sps*. showed resistance to positive control fluconazole. Quercetin exhibited the highest antifungal activity against *Alternaria sps*. with an inhibition zone of 23.1 mm diameter followed by *Fusarium oxysporum* (16.2 mm) and *Sclerotium hydrophilum* (11.6 mm).

This research evokes the efficacy of the methanol extract of *Euphorbia milii* stem in the control of diseases caused by pathogenic bacteria and fungi. Our results are justified by the earlier study reports of Senthamilselvi *et al*., **[20]**, who proposed that derivatives of quercetin have antimicrobial properties and the mode of action of these compounds were associated with their ability to form complexes with bacterial cell walls and extracellular soluble proteins.

Determination of MIC, MBC and MFC of pure compound:

The MIC and MBC values of a pure compound, quercetin isolated from *E. milii* stem are given in Table-3. The MIC values of quercetin were in the span of 0.78 mg/ml to 6.25 mg/ml and the MBC values were ranged between1.56mg/ml to 12.5 mg/ml. Quercetin exhibited low MIC value of 0.78 mg/ml against Gram positive test culture i.e., *Enterococcus faecalis*. The data on MIC and MFC of quercetin was recorded and tabulated **(Table 4)**. The data obtained through MIC showed some variation in inhibitory concentrations of the compound. The values of MIC against the tested fungi quercetin were observed to be within the

limit of 6.25 mg/ml to 12.5 mg/ml. This result suggests that bioactive compound isolated from stem of *E. milii* could be highly efficient for the treatment of several infectious ailments as the lower MIC and MBC values indicate higher efficacy as stated by Cowan *et al*. **[21]**.

Anticancer activity:

The cytotoxicity of the quercetin compound isolated from methanol extract of stem of *E. milii* was assessed using MTT cell proliferation assay **[22]** and Doxorubicin was used as a positive control. The quercetin exhibited positive correlation between the concentration of the compound (10 µM and 50 µM) and the percent inhibition of the growth of cell lines. All the cell lines were sensitive at 10 µM concentration but the % inhibition was different among the five cell lines used **(Figure 5)**. The most resistant cell line to quercetin was observed as a human alveolar adenocarcinoma cell line (A549) with 22.50% inhibition and the more sensitive cell line is the Human chronic myelogenous leukaemia cell line (K562) with 51.50% inhibition. The % inhibition of the human breast adenocarcinoma cell line (MCF-7) was 30.1 and that of the human cervical cancer cell line (HeLa) was 25.5.

The IC50 values of the compound of different cancer cell lines are presented in Table-5. From the results, it was observed that the quercetin showed excellent cytotoxic activity towards the examined cancer cell lines A549, Hela, MCF-7 and K562 cells. Quercetin exhibited relatively good and more cytotoxic action with IC50 value of 18.6µM against both MCF-7 carcinoma cells and K562 carcinoma cells. The results obtained were in concurrence with previous research findings of Abdur Rauf *et al*. **[23]** who reported that the crude methanolic extract of aerial parts of *E. milii* exhibited promising cytotoxic effects at different concentrations i.e., 10,100 and 1000µg/ml due to the presence of polar chemical compounds. However, the quercetin compound was found to be nontoxic without any inhibitory effect on cell proliferation against normal HEK-293 cells with only an insignificant decrease in cell survivability.

Phase contrast microscopy study of the quercetin treated cancer cells demonstrated the morphological changes like cell shrinkage and concentration-dependent detachment of non-viable cells on the surface of culture plate representing its anticancer activity (Plates – 2 to 6). Quercetin has been recorded to maintain anticancer properties by inhibiting the oncogene expression, such as c-fos, c-jun and ras **[24]**. The present research findings confirmed the anticancer

activity of quercetin against the tested carcinoma cell lines. Previous studies reported that the quercetin exhibited cytotoxicity in several human cancer cell lines, with diverse sensitivity **[25, 26]**.

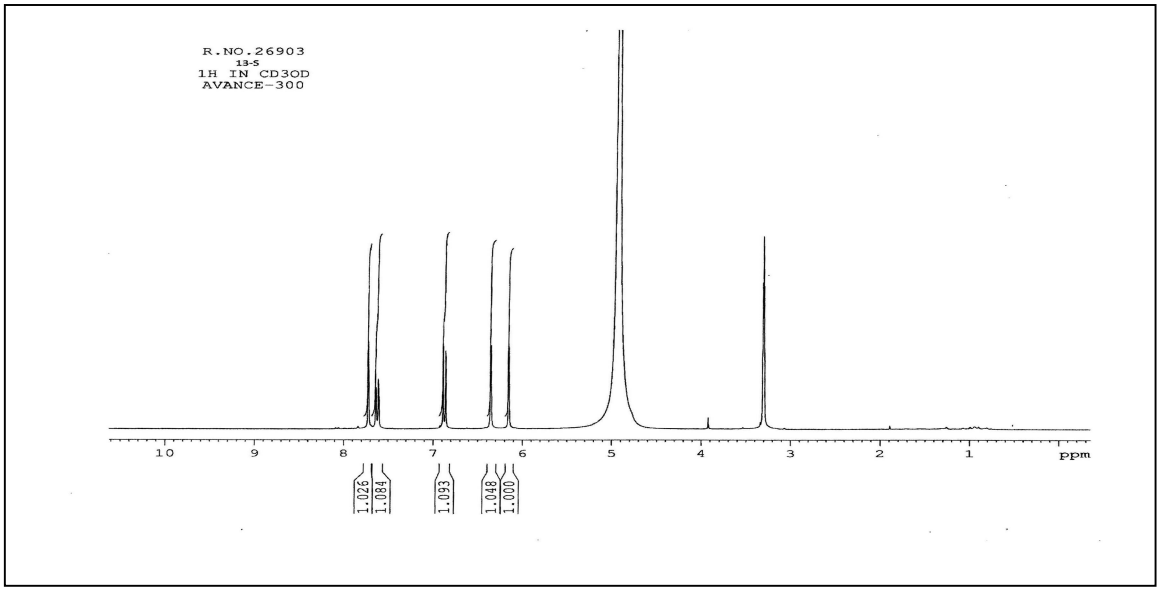


Figure 1: ^{1}H NMR spectrum of the pure compound isolated from stem extract of *E. milii*

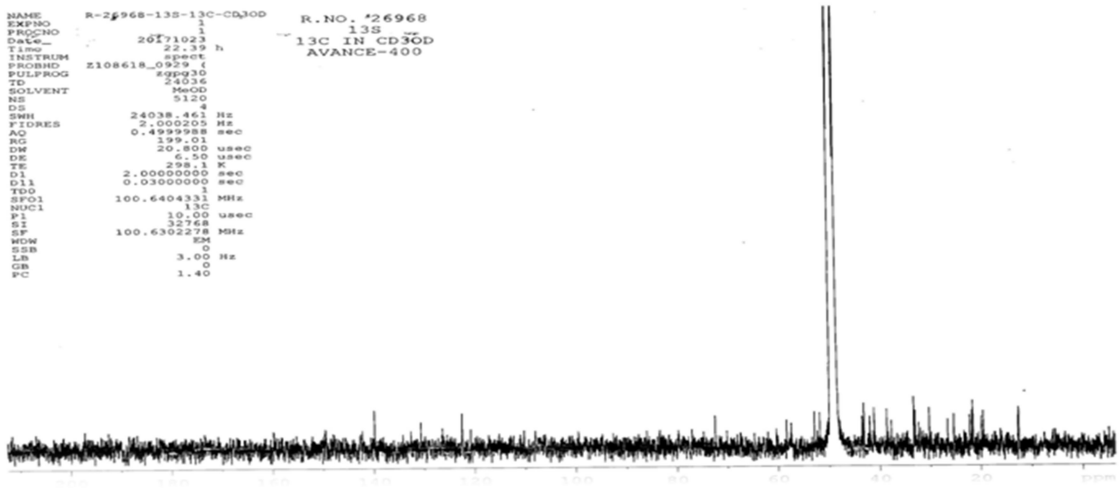


Figure 2: ^{13}C NMR spectrum of the pure compound isolated from stem extract of *E. milii*

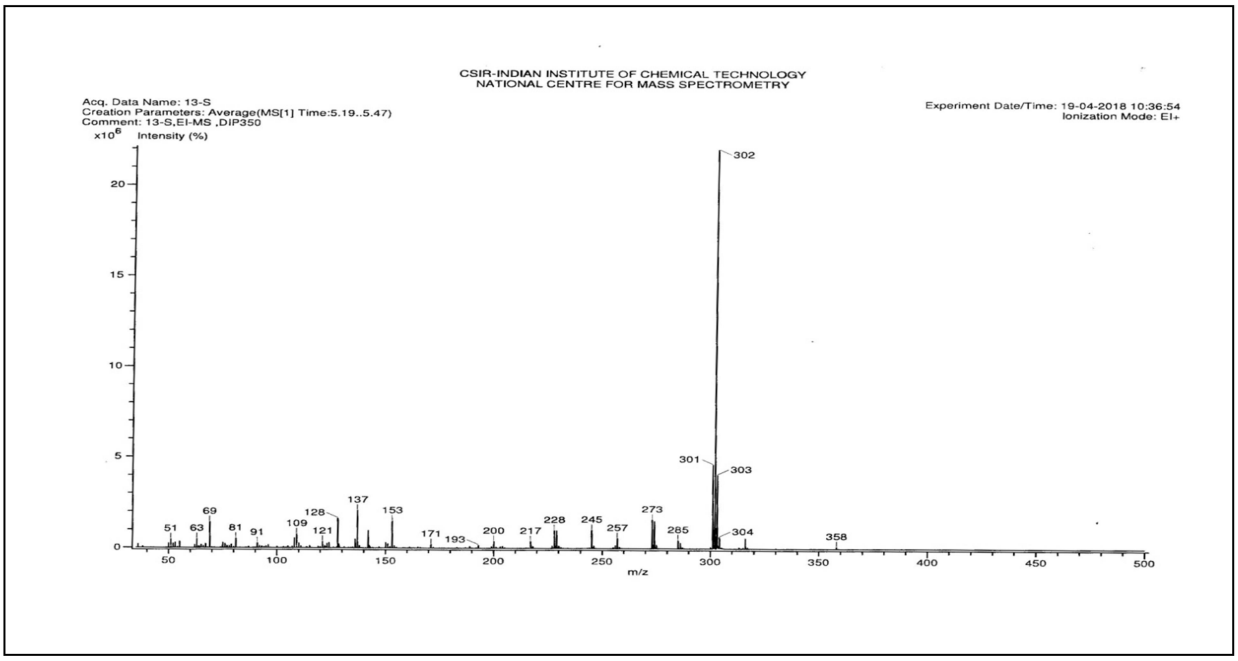


Figure 3: Mass spectrum of the pure compound isolated from stem extract of *E. milii*

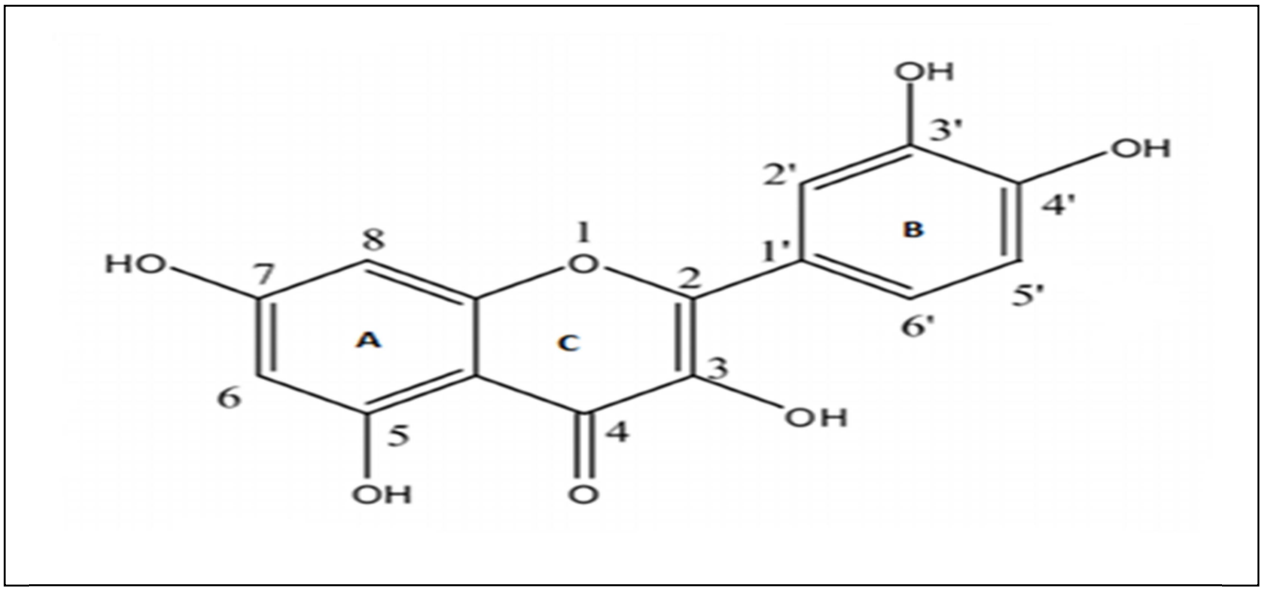


Figure 4: Chemical structure of the isolated quercetin (3,3',4',5,7-pentahydroxy-flavone)

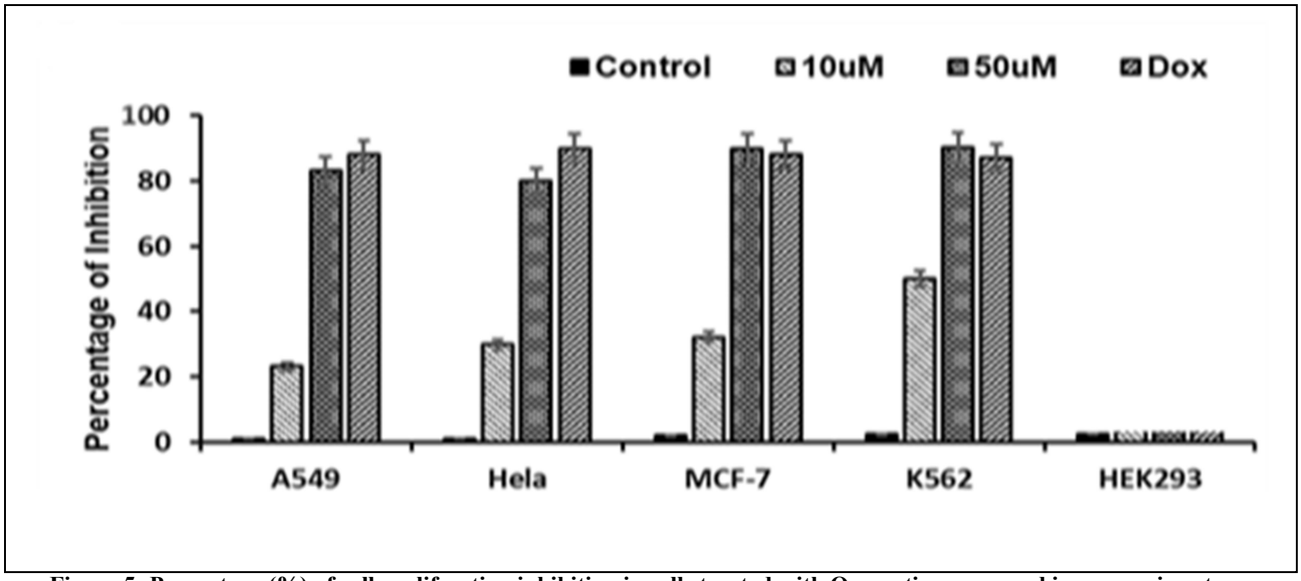


Figure 5: Percentage (%) of cell proliferation inhibition in cells treated with Quercetin compound in comparison to doxorubicin

Table 1: Antibacterial activity (Zone of inhibition) of Quercetin isolated from *E. milii* Stem

Test organisms	Zone of inhibition (mm)	
	Quercetin	Positive control (Streptomycin)
Micrococcus luteus MTCC 106	14.6±0.1	19.2±0.2
Arthrobacter protophormiae MTCC 2682	13.5±0.2	13.3±0.4
Rhodococcus rhodochrous MTCC 265	--	--
Bacillus megaterium MTCC 428	15.4±0.2	13.0±0.2
Bacillus subtilis MTCC 441	12.4±0.2	13.2±0.5
Enterococcus faecalis MTCC 439	14.1±0.1	13.3±0.2
Streptococcus mutans MTCC 497	12.1±0.2	14.0±0.2
Staphylococcus aureus MTCC 737	14.6±0.1	12.2±0.2
Lactobacillus acidophilus MTCC 10307	13.8±0.2	15.4±0.3
Alcaligens faecalis MTCC 126	12.8±0.1	11.1±0.2
Proteus mirabilis MTCC 425	10.8±0.2	12.2±0.2
Proteus vulgaris MTCC 426	13.8±0.5	13.4±0.3
Enterobacter aerogenes MTCC 10208	--	--
Salmonella enterica MTCC 3858	15.2±0.3	11.2±0.2
Pseudomonas aeruginosa MTCC 1688	16.2±0.1	13.0±0.1

Table 2: Antifungal activity (Zone of inhibition) of Quercetin isolated from *E. milii* Stem

Test organisms	Zone of inhibition (mm)	
	Quercetin	Positive control (Fluconazole)
Fusarium oxysporum MTCC 7392	16.2±0.1	--
Sclerotium hydrophilum MTCC 2157	11.6±0.4	25.2±0.3
Alternaria sps. MTCC 9692	23.1±0.2	--

Table 3: MIC and MBC values (mg/ml) of Quercetin isolated from *E. milii* stem extract

Test Organisms	Quercetin	
	MIC	MBC
Micrococcus luteus MTCC 106	6.25	12.5
Arthrobacter protophormiae MTCC 2682	12.5	25.0
Rhodococcus rhodochrous MTCC 265	--	--
Bacillus megaterium MTCC 428	3.12	6.25
Bacillus subtilis MTCC 441	6.25	12.5
Enterococcus faecalis MTCC 439	0.78	1.56
Streptococcus mutans MTCC 497	12.5	25.0
Staphylococcus aureus MTCC 737	3.12	6.25
Lactobacillus acidophilus MTCC 10307	6.25	12.5
Alcaligenes faecalis MTCC 126	1.56	3.12
Proteus mirabilis MTCC 425	6.25	12.5
Proteus vulgaris MTCC 426	3.12	6.25
Enterobacter aerogenes MTCC 10208	--	--
Salmonella enterica MTCC 3858	1.56	3.12
Pseudomonas aeruginosa MTCC 1688	1.56	3.12

Table 4: MIC and MFC values (mg/ml) of Quercetin isolated from *E. milii* stem extract

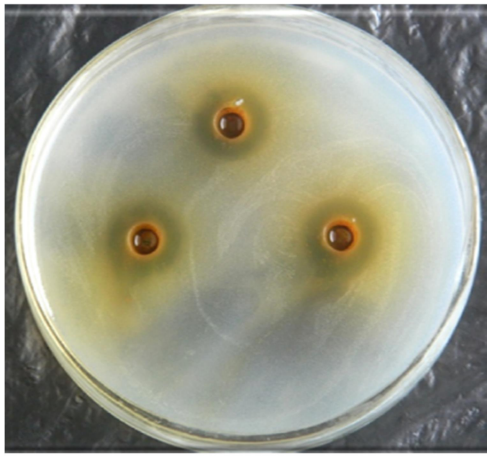
Test organisms	Quercetin	
	MIC	MFC
Fusarium oxysporum MTCC 7392	12.5	25.0
Sclerotium hydrophilum MTCC 2157	12.5	25.0
Alternaria sps. MTCC 9692	6.25	12.5

Table 5: IC_{50} values of quercetin and doxorubicin on different cancer cell lines

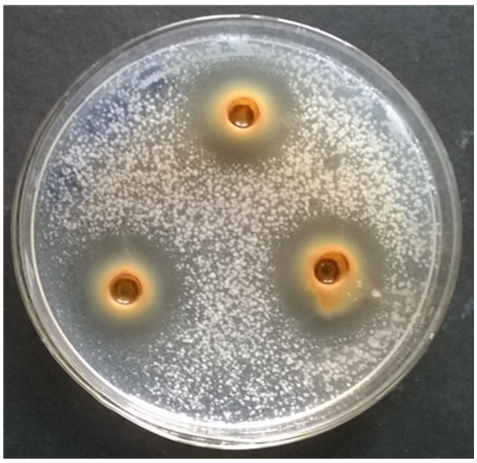
Compounds	IC_{50}values (µM)				
	A549	Hela	MCF-7	K562	HEK293
Quercetin	24.70 ± 1.29	22.10 ± 1.6	18.60 ± 1.5	18.60 ± 1.9	-
Doxorubicin	0.75 ± 0.28	0.9 0± 0.45	0.84 ± 0.30	0.91 ± 0.45	-

Values given are the mean of triplicates ± Standard deviation

–[a] No activity; A549- Human alveolar adenocarcinoma cell line; HeLa- Human Cervical cancer cell line; MCF-7 - Human breast adenocarcinoma cell line; K562 - Human chronic myelogenous leukemia cell line; HEK 293 - Human embryonic kidney cell line



Quercetin against *Proteus mirabilis*



Quercetin against *Fusarium oxysporum*

Plate 1: Antimicrobial activity (zone of inhibition) of purified Quercetin compound

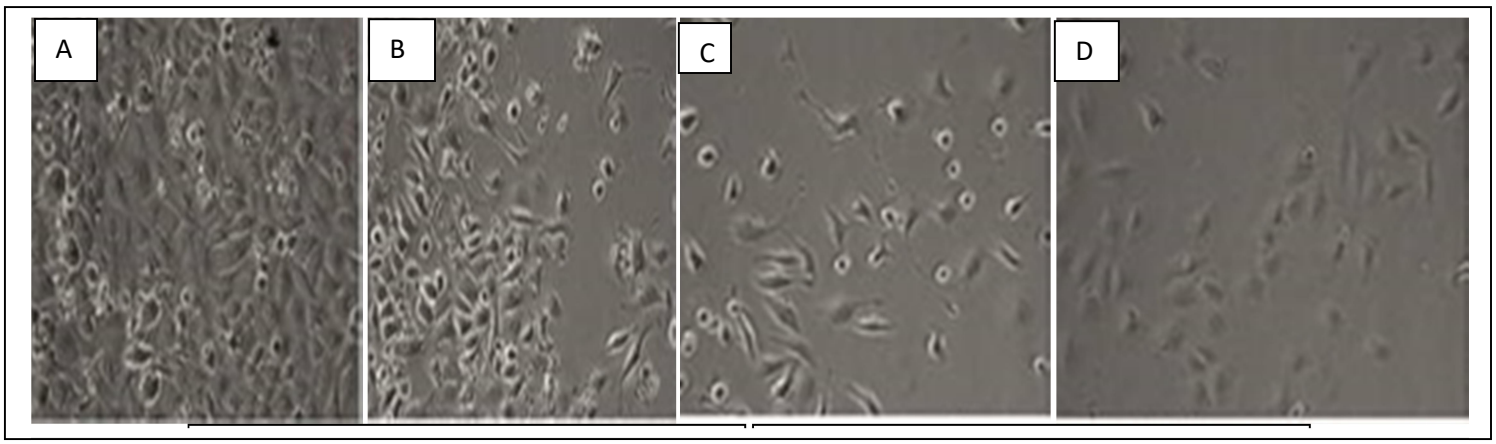


Plate 2: Cytotoxicity of quercetin on A 549 cell line

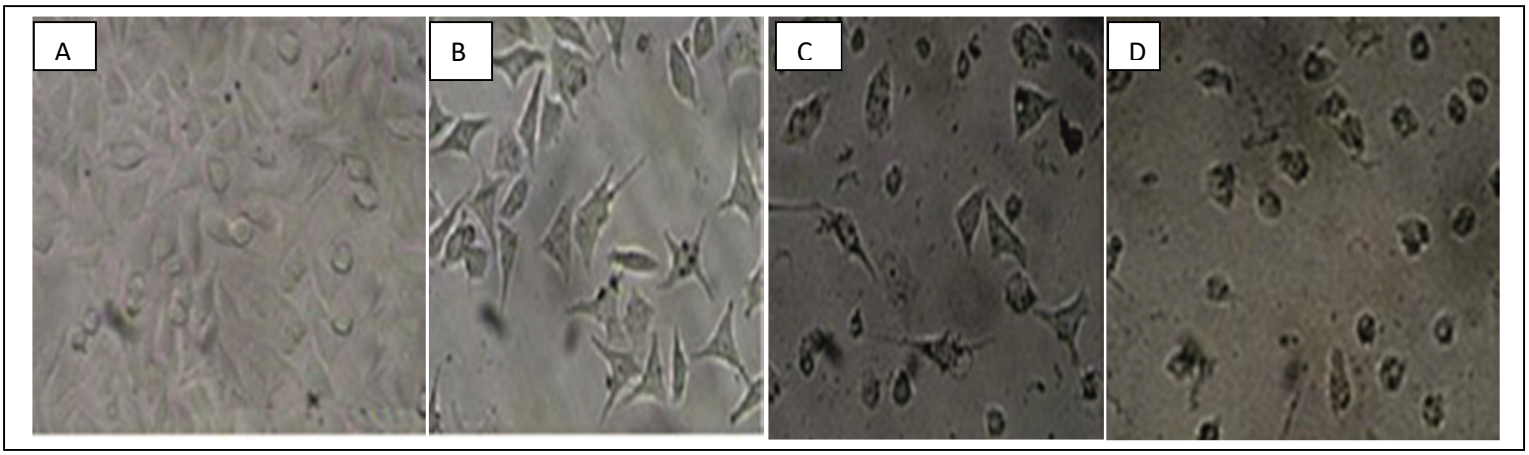


Plate 3: Cytotoxicity of Quercetin on HeLa cell line

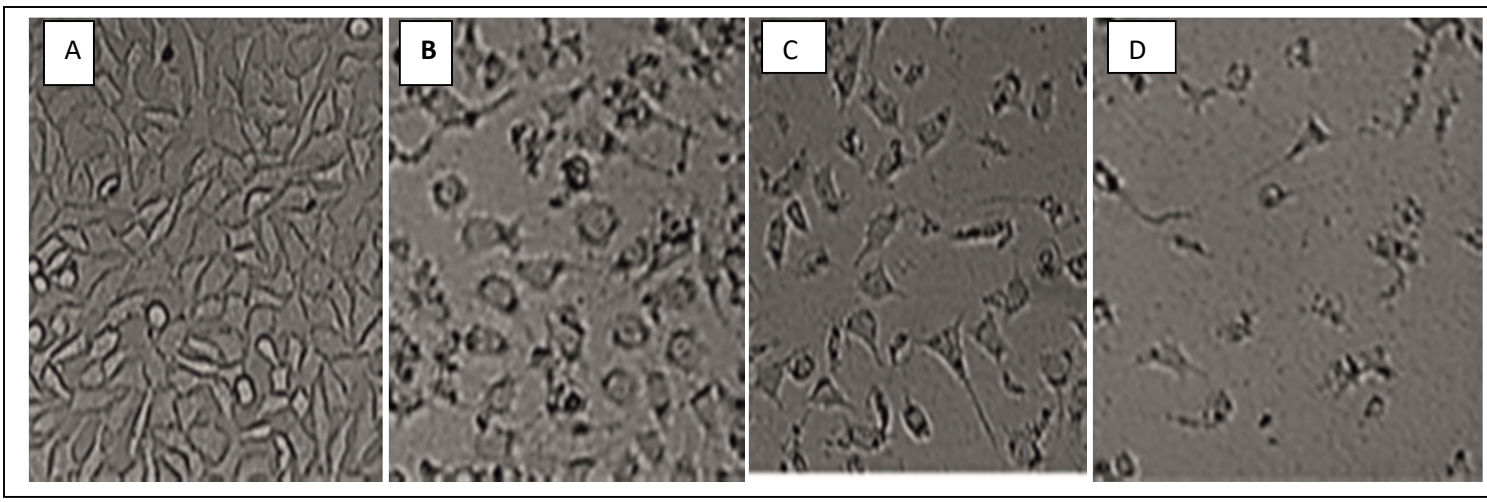


Plate 4: Cytotoxicity of Quercetin on MCF-7 cell line

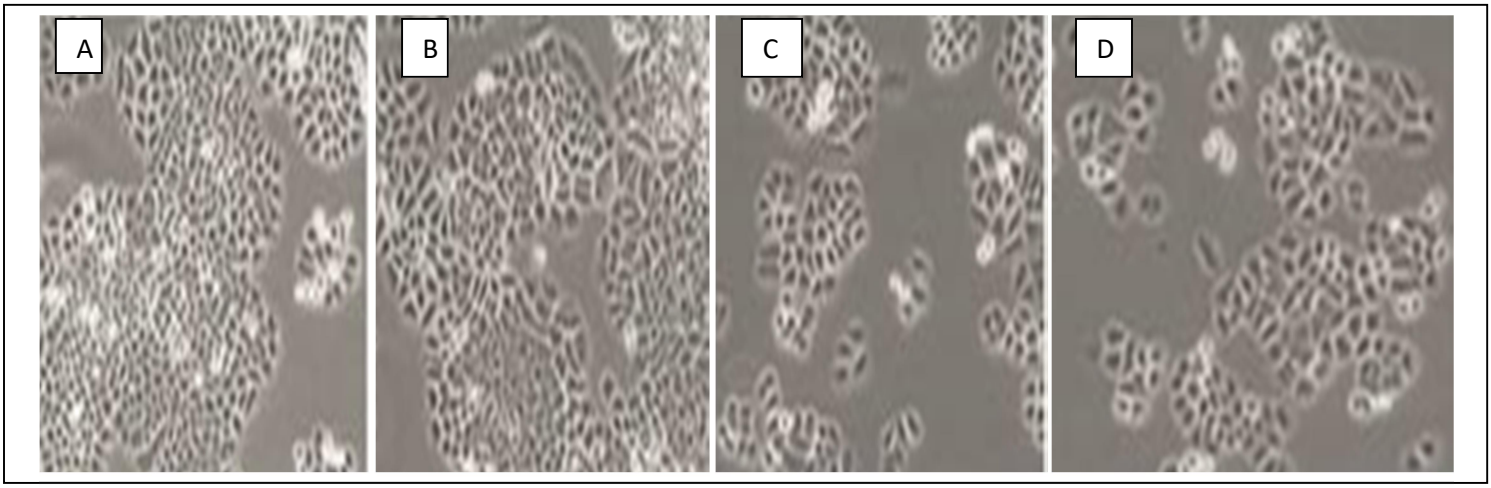


Plate-5: Cytotoxicity of Quercetin on K 562 cell line

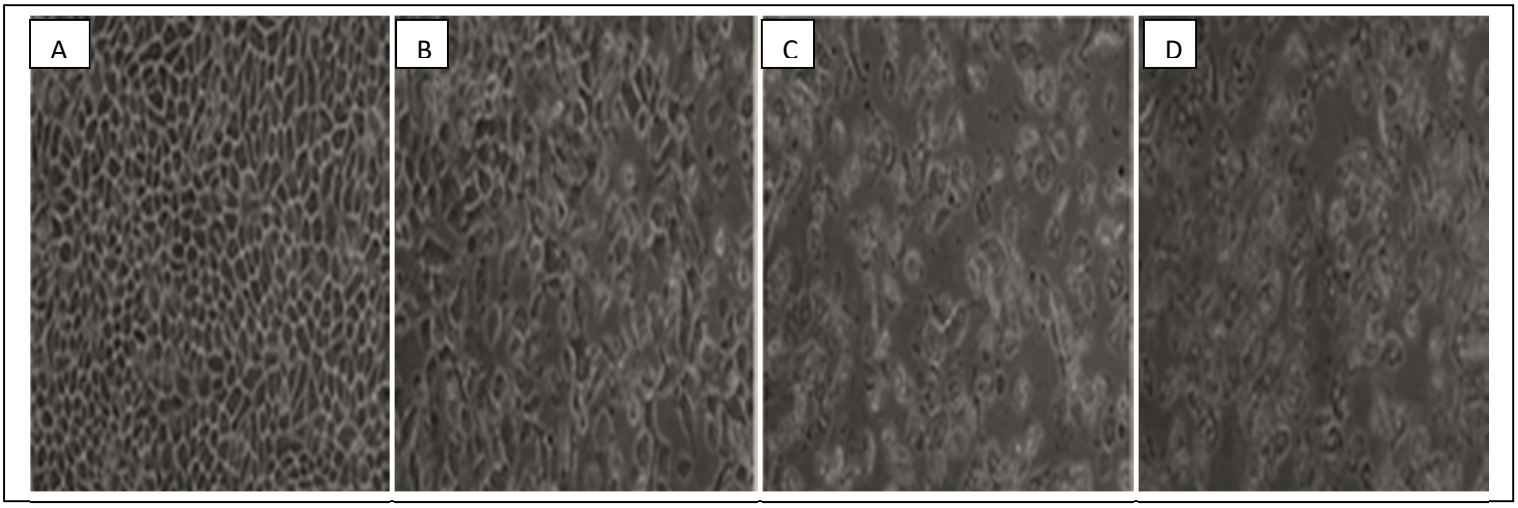


Plate 6: Cytotoxicity of Quercetin on HEK-293 cell line
A) Untreated cells; B) Cells treated with 10 µM quercetin; C) Cells treated with 50 µM quercetin; D) Cells treated with standard drug 1µM doxorubicin

CONCLUSION

Results indicate that quercetin isolated from methanol extract of *E. milii* stem is an effective compound with a broad spectrum of curative applications. This was the first report on isolation and characterization of quercetin compound from methanol extract of *E. milii* stem. The identification of pure compound with antimicrobial and anticarcinogenic properties from stem extract of this plant indicates that *E. milii* is an exciting source for interesting bioactive molecules that can be future promise as an alternative resource of drugs in the pharmaceutical industry.

REFERENCES

[1] Dayan, F. E., & Duke, S. O. (2009). Biological activity of allelochemicals.

In *Plant-derived Natural Products*; pp. 361-384, Springer, New York, NY.

[2] Osbourn, Anne.E, Lanzotti, V. (2009), Plant-Derived Natural Products: Synthesis, Function, and Application, 1st ed.; Springer: New York, NY, USA.

[3] Van Wyk, B. E., & Wink, M. (2018). *Medicinal plants of the world*. CABI.

[4] Chintamunnee, V., & Mahomoodally, M. F. (2012). Herbal medicine commonly used against non-communicable diseases in the tropical island of Mauritius. *Journal of Herbal Medicine*, *2*(4), 113-125.

[5] Horn, J. W., van Ee, B. W., Morawetz, J. J., Riina, R., Steinmann, V. W., Berry, P. E., & Wurdack, K. J. (2012). Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution*, *63*(2), 305-326.

[6] Marie Cris D. Gapuz, Ronnie L. Besagas (2018). Phytochemical profiles and antioxidant activities of leaf extracts of euphorbia species. *Journal of Biodiversity and environmental Sciences,* 12(4), 59-65.

[7] Bajpai, V. K., Majumder, R., & Park, J. G. (2016). Isolation and purification of plant secondary metabolites using column-chromatographic technique. *Bangladesh Journal of Pharmacology*, *11*(4), 844-848.

[8] Al Habsi, A. A. S., & Hossain, M. A. (2018). Isolation, structure characterization and prediction of antioxidant activity of two new compounds from the leaves of *Dodonaea viscosa* native to the Sultanate of Oman. *Egyptian journal of basic and applied sciences*, *5*(2), 157-164.

[9] Gu, W. Z., Chen, R., Brandwein, S., McAlpine, J., & Burres, N. (1995). Isolation, purification, and characterization of immuno-suppressive compounds from tripterygium: triptolide and tripdiolide. *International journal of immunopharmacology*, *17*(5), 351-356.

[10] Kakar, M., Amin, M. U., Alghamdi, S., Sahibzada, M. U. K., Ahmad, N., & Ullah, N. (2020). Antimicrobial, Cytotoxic, and Antioxidant Potential

of a Novel Flavone "6, 7, 4′-Trimethyl Flavone" isolated from *Wulfenia amherstiana*. *Evidence-Based Complementary and Alternative Medicine*, Volume 2020, 12 pages.

[11] Rasul, Mohammed Golam (2011). Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. *International Journal of Basic Sciences and Applied Computing*, *2*(6), 1-6.

[12] Van der Watt, E., & Pretorius, J. C. (2001). Purification and identification of active antibacterial components in *Carpobrotus edulis* L. Journal of Ethnopharmacology, *76*(1), 87-91.

[13] Christophoridou, S., Dais, P., Tseng, L. H., & Spraul, M. (2005). Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with postcolumn solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). Journal of agricultural and food chemistry, 53(12), 4667-4679.

[14] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), 71-79.

[15] Joray, M. B., Del Rollán, M. R., Ruiz, G. M., Palacios, S. M., & Carpinella, M. C. (2011). Antibacterial activity of extracts from plants of central Argentina—isolation of an active principle from *Achyrocline satureioides*. *Planta medica*, *77*(01), 95-100.

[16] Sahranavard SH, Naghibi FA, Ghaffari SA. (2012), Cytotoxic activity of extracts and pure compounds of Bryonia aspera. Int J Pharm Pharmaceut Sci. 4(3): 541-3.

[17] Lakhanpal, P., & Rai, D. K. (2007). Quercetin: a versatile flavonoid. Internet Journal of Medical Update, 2(2), 22-37.

[18] Kamurthy, H., Dontha, S., & Rajani, K. (2015). Phytochemical screening on *Euphorbia milii*red flowers-Isolation of terpenoids, flavone and phenols. American Journal of Ethnomedicine, 2(6), 322-32

[19] Gobbo-Neto, L., & Lopes, N. P. (2007). Medicinal plants: factors of influence on the content of

secondary metabolites, Quim. Nova, 30 (2). 374-381.

[20] Senthamilselvi, M. M., Kesavan, D., & Sulochana, N. (2012). An anti-inflammatory and anti-microbial flavone glycoside from flowers of *Cleome viscosa*. Organic and medicinal chemistry letters, 2(1), 19.

[21] Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582.

[22] Ukwubile, C. A., Ikpefan, E. O., Malgwi, T. S., Bababe, A. B., Odugu, J. A., Angyu, A. N., & Nettey, H. I. (2020). Cytotoxic effects of new bioactive compounds isolated from a Nigerian anticancer plant *Melastomastrum capitatum* Fern. leaf extract. *Scientific African*, *8*, e00421.

[23] Umesalma, S., Nagendraprabhu, P., & Sudhandiran, G. (2015). Ellagic acid inhibits proliferation and induced apoptosis via the Akt signaling pathway in HCT-15 colon adenocarcinoma cells. Molecular and cellular biochemistry, 399(1-2), 303-313.

[24] Rauf, A., Imran, M., Khan, I. A., ur-Rehman, M., Gilani, S. A., Mehmood, Z., & Mubarak, M. S. (2018). Anticancer potential of quercetin: A comprehensive review. Phytotherapy Research, *32*(11), 2109-2130.

[25] Abdur Rauf, Ajmal Khan, Nizam Uddin, Roohullah. (2013). "cytotoxic study of aerial parts of *E.milii* and *E.pulcherrima"*, Topclass Journal of Herbal Medicine Vol. 2(12), 266-269.

[26] Chen, F. Y., Cao, L. F., Wan, H. X., Zhang, M. Y., Cai, J. Y., Shen, L. J., & Zhong, H. (2015). Quercetin enhances adriamycin cytotoxicity through induction of apoptosis and regulation of mitogen-activated protein kinase/extracellular signal-regulated kinase/c-Jun N-terminal kinase signaling in multidrug-resistant leukemia K562 cells. *Molecular medicine reports*, 11(1), 341-348.