



# Research Journal of **Microbiology**

ISSN 1816-4935



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)



## Research Article

# Antimicrobial Activity of *Moringa concanensis* Flower Against Human Pathogens and its Cytotoxic Effects on HepG2 Cell Line

<sup>1</sup>Sivakumar Duraikannu, <sup>2</sup>Manikandan Rengasamy and <sup>3</sup>Velu Periyannan

<sup>1</sup>Department of Biotechnology, Bharathidasan University Constituent College, Perambalur, 621107, Tamilnadu, India

<sup>2</sup>Department of Microbiology, Bharathidasan University Constituent College, Perambalur, 621107, Tamilnadu, India

<sup>3</sup>SCIGEN Research and Innovation Pvt. Ltd., Periyar Technology Business Incubator, Periyar Nagar, Thanjavur, 613403, Tamilnadu, India

## Abstract

**Background and Objective:** The microbial infection is a foremost cause of mortality worldwide owing to the emergence of multidrug-resistant pathogens. *Moringa concanensis* is an imperative medicinal plant that was extensively utilized to treat the numerous ailments in India. The current research investigation was intended to screen the phytochemical profile and examine the antimicrobial and cytotoxic potentials of *M. concanensis* flowers. **Materials and Methods:** The pharmacognostic and phytochemical screenings were done by adopting the standard procedures. The antimicrobial efficiency of *M. concanensis* flower extracts was inspected through the disk diffusion technique. The Minimum Bactericidal and Fungicidal Concentration (MBC and MFC) was investigated through the tube dilution technique. The MTT cytotoxic assay was executed to inspect the cytostatic activity of chloroform extract of *M. concanensis* flower. **Results:** The results of phytochemical screening and fluorescence assay were proved the presence of numerous phytoconstituents in the flowers of *M. concanensis*. The outcomes of antimicrobial studies displayed that the *M. concanensis* flowers have potent antimicrobial action against the clinical pathogens. The chloroform extract of the *M. concanensis* flower possessed a noticeable cytotoxic potential to the human lung cancer (HepG2) cell line. **Conclusion:** The findings of this investigation is the evidence that the flowers of *M. concanensis* have potent antimicrobial benefits and cytostatic effects against HepG2 cells.

**Key words:** Liver cancer, phytochemical analysis, pharmacognostic study, *M. concanensis* flowers, antimicrobial activity

**Citation:** Sivakumar Duraikannu, Manikandan Rengasamy and Velu Periyannan, 2020. Antimicrobial activity of *Moringa concanensis* flower against human pathogens and its cytotoxic effects on HepG2 cell line. Res. J. Microbiol., 15: 51-60.

**Corresponding Author:** Sivakumar Duraikannu, Department of Biotechnology, Bharathidasan University Constituent College, Perambalur, 621107, Tamilnadu, India

**Copyright:** © 2020 Sivakumar Duraikannu *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The global impact of infectious ailments is spoiling the life quality of individuals and becoming a life intimidating problem. Presently, the healing of infectious ailments has become the biggest problem owing to the raising of multidrug-resistant pathogens. There are plentiful causes behind the emerging of antibiotic-resistant pathogens, but the frequent one is the improper and limitless utilization of antibiotics<sup>1</sup>. The excessive utilization of numerous antibiotics has triggered the rapid emergence of multi-drug resistant pathogens. The morbidity as well as mortality, hospitalization durations, healthcare complications and economical burdens were raised in recent decades that owing to the multi-drug resistant pathogens. The microbial infections were regarded as a major public health problem worldwide in this century<sup>2</sup>. In case of fungal infection, numerous kinds of anti fungal drugs were available and on the other hand, the raising of multi-drug resistance is hugely hindering the treatment to the infected patients. Among these resistant fungal species the *Candida* and *Aspergillus* species displayed an augmented resistance towards the many kinds of drugs. As a result of multi-drug resistant especially the *Candida* species is a prime challenge to the clinical success rate of infected patients<sup>3</sup>.

The microbial infectious ailments are unevenly affecting the individuals worldwide, particularly in developing and under-developing countries. For this magnificent prevalence of microbial infections is not only due to the microbes, but also owing to the lack of hygiene among peoples, poor health infrastructure, counterfeit drugs, vaccine errors and health awareness deficits. The preceding research findings were highlighted that numerous bacterial strains were displayed a marked resistance towards the different antibiotics<sup>4</sup>. For this reason, the urgent need to explore the herbal plants to identify the novel, inexpensive, potential compounds as an alternative approach to the antibiotics utilization<sup>4</sup>. Since the pre-historic periods the natural products were extensively utilized to heal the countless ailments particularly infectious ailments. The herbal based natural products especially the medicinal plant extracts are conventional reserves for the discovery of novel therapeutic agents to heal the ailments<sup>5,6</sup>. Cancer was holding the top place in causing frequent deaths worldwide, at present, the cancer was treated via many routes for instance, surgical elimination of tumors, chemotherapy, radiotherapy and medications but those are often failed to augment the well beings of sarcoma victims in many cases ends with death<sup>7</sup>. Even though the liver sarcoma was well treated via those approaches like surgical exclusion, radiation and medications but the success rate was

shockingly low and failed to regain the well beings of victims in that way, less than 15% liver cancer victims were benefited surviving up to 5 years or more<sup>8</sup>.

It was reported that almost 20 kinds of human illnesses can be treated via utilizing the *M. concanensis* plant. Preceding report highlighted that the *M. concanensis* was extensively consumed to heal the skin tumor, tiredness, to reduce blood pressure, aphrodisiac, jaundice, eye-care, diabetes and bloat<sup>9</sup>. The preceding research findings have revealed that the plant *M. concanensis* possessed greater biological benefits like anti-inflammatory potential, antianalgesic, antipyretic, antiepileptic, antioxidant activity and sunscreen<sup>10-12</sup>. Conversely there are few explorations were done on the flowers of *M. concanensis* to claim their antimicrobial, cytotoxic and free radical scavenging potentials. Consequently, in this research exploration, it was intended to examine the *M. concanensis* flowers on cytotoxic activity and antimicrobial potential against the clinical pathogens.

## MATERIALS AND METHODS

**Study area:** The study was carried out at the Department of Biotechnology and Department of Microbiology, Bharathidasan University Constituent College from September-December, 2019.

**Chemicals:** The Dulbecco's Modified Eagles Medium (DMEM) medium, Fetal Bovine Serum (FBS), Penicillin/Streptomycin, Mueller Hinton Agar (MHA) medium, Sabouraud Dextrose Agar (SDA) medium, Ciprofloxacin and Fluconazole were acquired in Sigma-Chemical Co., St. Louis, USA. The other entire chemicals and reagents that were utilized in this investigation are of diagnostic grade acquired from Hi-Media Lab, Virginia, USA.

**Collection and authentication of plant material:** The healthy and matured flowers of *M. concanensis* were gathered from the Esanai village, Perambalur, Tamilnadu, India (Latitude 11.2982°N, Longitude 78.8298°E) during September, 2019. The collected plant specimen was scientifically identified and authenticated by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli-620002, Tamilnadu, India. The voucher specimen number is SK001.

**Preparation of *M. concanensis* flower powder:** The collected healthy flowers of *M. concanensis* were dehydrated in a shady cabin at room temperature. Then the dehydrated

flowers were uniformly grounded using a mechanical grinder to yield fine powder. Finally, the powdered flowers were utilized to extract preparation.

**Preparation of *M. concanensis* flower extracts:** The 15 g of powdered flowers were soaked separately in 100 mL chloroform, diethyl ether and petroleum ether, maintained 2 days at 37°C with occasional stirring. After that, the suspension was sifted with the aid of clean muslin cloth subsequently Whatman No. 1 sift paper. The resulted filtrate was then processed on a rotary evaporator fitted with the vacuum pump. Finally, the extract was stored at 4°C until further utilization.

**Fluorescent analysis:** A small quantity of dehydrated powder and extract of flowers of *M. concanensis* were located on a slide separately and then 1-2 drops of freshly prepared reagents were mixed with gentle tilting for 1-2 min. Then the slides were examined in day (visible) light and placed it to the viewing chamber of UV transilluminator and examined in both short UV (254 nm) and long UV (365 nm) radiations. The observed colors via mixing the powder and extract with diverse reagents in several radiations were noted and tabulated<sup>13</sup>.

**Qualitative phytochemical screening:** The each extracts were inspected through diverse qualitative phytochemical investigations to establish the phytochemical profile of the *M. concanensis* flowers. All three extracts were inspected to evidence the occurrence of various phytoconstituents via standard procedure<sup>14,15</sup>.

**Cell culture:** The human liver cancer (HepG2) cells were bought in American Type Culture Collection (ATCC), USA. The cells were then cultured in DMEM with 10% FBS and 1% Penicillin-Streptomycin mixture and maintained at 37°C in a moistened atmosphere containing 5% CO<sub>2</sub> and 95% air incubation.

**MTT cytotoxic assay of *M. concanensis* flowers:** The cytotoxicity action of chloroform extract of *M. concanensis* flowers were examined via the MTT cytotoxic test<sup>16</sup>. The HepG2 cell lines were loaded in the 96-well plates in a population of  $1 \times 10^4$  cells per well and kept incubation for 24 h at 37°C. To elucidate the cytotoxic potential of chloroform extract of *M. concanensis* flowers it was dissolved in DMSO and supplemented in different dosages (10-100 µg mL<sup>-1</sup>) to the HepG2 cells and then kept at

incubation for 24 h at 37°C. After 24 h of incubation, the MTT solution (100 µL from 5 mg mL<sup>-1</sup>) was loaded into every well and then plates were again kept for incubation for 4 h. Then the medium was eliminated from the wells and 100 µL of the serum free medium was replenished into the wells and the developed formazan crystals were liquefied by the addition of dimethyl sulfoxide. Finally, the absorbance value was determined via multi well microplate reader at 570 nm.

**Morphological analysis of chloroform extract of *M. concanensis* flowers treated HepG2 cells:** To examine the chloroform extract of *M. concanensis* flowers treated HepG2 cells, the HepG2 cells were loaded on the 96-well plate at  $1 \times 10^5$  cell population per well. Then the HepG2 cells were supplemented with 25-100 µg mL<sup>-1</sup> of chloroform extract of *M. concanensis* flowers and incubated for 24 h. Later after incubation, HepG2 cells were inspected beneath the optical microscope to identify the extract induced morphological alterations<sup>17</sup>.

**Collection and maintenance of bacterial and fungal strains:** The bacterial strains i.e., *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and fungal strains i.e., *Aspergillus flavus*, *Candida albicans*, *Trichophyton simii*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* were acquired from the ATCC, USA. The bacterial and fungal strains were maintained individually in MHA medium at 38°C for bacteria and SDA medium at 28°C for fungi in an incubator and utilized for further investigations.

**Examination of antimicrobial potential of *M. concanensis* flowers via disc diffusion technique:** The antimicrobial potential of *M. concanensis* flowers were investigated through the disk diffusion technique<sup>18</sup>. Briefly, 100 µL of 24 h old culture of every bacterial strain were loaded to the MHA medium fungal strains were SDA medium and spread over the surface of plates. Then the sterile antibiotic discs in 6 mm diameter were infused with each extracts of *M. concanensis* flowers then the discs were located on a test plates at equidistance. The plates were then kept undisturbed for 30 min to diffuse the extract on the medium and then incubated at 37°C for 24 h to examine the antimicrobial potential of *M. concanensis* flowers. Ciprofloxacin antibiotic discs (15 µL/disc) were utilized as a reference control for bacteria and Fluconazole discs (15 µL/disc) were utilized for fungi. Later than the incubation the zone of inhibition (in mm) around the disc were examined and regarded as an antimicrobial efficacy of extracts against the test pathogens.

### Determination of Minimum Bactericidal and Fungicidal Concentrations (MBC and MFC) of *M. concanensis* flowers:

The Minimum Inhibitory Concentration (MIC) is the least dose needed to hinder the microbial growth subsequently 24 h incubation. The MIC examination through the tube dilution technique was extensively employed to inspect the antimicrobial potential of test sample<sup>19</sup>. The growth sensitivity of bacterial and fungal pathogens to the *M. concanensis* flower extracts was examined via the tube dilution technique to calculate the MBC and MFC. Briefly, strains were loaded to the tube consisting of 1 mL of respective broth at the density of  $5 \times 10^5$  CFU mL<sup>-1</sup> of each test pathogens. Then the tube was supplemented with the various doses (100-1000 µg mL<sup>-1</sup>) of *M. concanensis* flower extracts. The tubes were further diluted with the addition of MH broth for bacteria and SD broth for fungi. Finally, the assay tubes consisting of diluted samples were then sustained overnight at their respective temperatures for bacteria and fungi with constant shaking. The growth of the strains was inspected via the turbidity formation. The clear tubes excluding turbidity denote the absence of bacterial and fungal growth. The extra care was taken to make sure the sterility and this investigation was repeated for thrice to establish the precise test values<sup>20,21</sup>. Later than the completion of the investigation, the utilized microbial strains, growth medium and every plastic ware were sterilized and discarded as per the rules suggested by the Institutional Biosafety Committee (IBC).

**Statistical examination:** Statistical investigations were carried out with the help of the SPSS statistical tool (version 16). Data were examined through ANOVA subsequently DMRT study to distinguish the variations among the test groups. Data were displayed as Mean  $\pm$  SD of triplicate measurement. Data were regarded as significant if 'p' values were less than 0.05.

## RESULTS

**Characteristics of *M. concanensis* flower powder:** The dehydrated and powdered flowers were brownish and have a characteristic flavor in nature. The flower powder has a defined and solid aroma. The greasy spots were noted while the powder was mechanically pressed along with the filter papers. It eventually denotes the occurrence of fatty acids. The stable froth also monitored during the powder was mixed with water and shaken well. It denotes the occurrence of saponins in the flower.

**Fluorescence analysis of *Moringa concanensis* flower extracts and powder:** The outcomes of fluorescence analysis of *M. concanensis* flower extracts as well as powder, evidently proved that this plant has the immense phytoconstituents. The results displayed the different kinds of fluorescent colors like brown, greenish-brown, violet, green, purple, orange and black when exposed to the sun (day) light and UV light (Table 1). The appearance of numerous kinds of color fluorescence was proving the occurrence of an array of phytoconstituents in the *M. concanensis* flowers (Table 2).

**Qualitative phytochemical analysis of *M. concanensis* flower:** As depicted in Table 3, the outcome of the qualitative phytochemical examination of *M. concanensis* flower has displayed the occurrence of numerous kinds of phytochemicals. The flowers displayed the occurrence of flavonoids, alkaloids, glycerides, volatile oils, steroids, terpenoids, glycosides, reducing sugars and amino acids. While comparing to other solvents, the chloroform extract of *M. concanensis* flower displayed the presence of a maximum

Table 1: Fluorescence analysis of *M. concanensis* flower extracts

| Chemical test                               | Chloroform extract |                 | Diethyl ether extract |                 | Petroleum ether extract |          |
|---------------------------------------------|--------------------|-----------------|-----------------------|-----------------|-------------------------|----------|
|                                             | Day light          | UV light        | Day light             | UV light        | Day light               | UV light |
| Extract+Aqu. NaOH 50%                       | Brownish black     | Violet          | Brown                 | Violet          | Orange                  | Violet   |
| Extract+Alc. NaOH 50%                       | Brown              | Violet          | Blackish brown        | Violet          | Brown                   | Purple   |
| Extract+Con. HCL                            | Greenish brown     | Black           | Green                 | Purple          | Pale red                | Purple   |
| Extract+50% HCL                             | Greenish black     | Dark purple     | Brown                 | Dark purple     | Brown                   | Purple   |
| Extract+Con. HNO <sub>3</sub>               | Brown              | Purple          | Pale brown            | Violet          | Orange                  | Black    |
| Extract+50% HNO <sub>3</sub>                | Brownish yellow    | Violet          | Greenish brown        | Black           | Reddish brown           | Black    |
| Extract+Con. H <sub>2</sub> SO <sub>4</sub> | Greenish black     | Purple          | Greenish yellow       | Blackish brown  | Black                   | Violet   |
| Extract+50% H <sub>2</sub> SO <sub>4</sub>  | Black              | Dark purple     | Violet                | Blackish purple | Reddish brown           | Violet   |
| Extract+Ammonia solution                    | Greenish black     | Dark violet     | Greenish brown        | Black           | Orange                  | Purple   |
| Extract+1% Iodine solution                  | Reddish brown      | Blackish violet | Purple                | Purple          | Red                     | Black    |
| Extract+10% FeCl <sub>2</sub>               | Brown              | Dark violet     | Brown                 | Violet          | Pale black              | Black    |
| Extract+Glacial acetic acid                 | Brown              | Violet          | Reddish brown         | Reddish brown   | Greenish yellow         | Black    |
| Extract+Ethanol                             | Greenish black     | Reddish brown   | Brown                 | Violet          | Brownish black          | Purple   |

Table 2: Results of fluorescence analysis of *Moringa concanensis* flower powder

| Reagents                                            | Day light       | Short UV   | Long UV       |
|-----------------------------------------------------|-----------------|------------|---------------|
| Powder+1 M H <sub>2</sub> SO <sub>4</sub>           | Greenish yellow | Black      | Black         |
| Powder+1 M HCl                                      | Greenish yellow | Violet     | Violet        |
| Powder+10% CuSO <sub>4</sub>                        | Green           | Violet     | Dark violet   |
| Powder+Con. HNO <sub>3</sub>                        | Reddish brown   | Brown      | Violet        |
| Powder+Dil. HNO <sub>3</sub>                        | Greenish yellow | Black      | Black         |
| Powder+Con. HNO <sub>3</sub> + Dil.HNO <sub>3</sub> | Reddish brown   | Purple     | Violet        |
| Powder+10% NaOH                                     | Green           | Violet     | Dark violet   |
| Powder+1% Glacial acetic acid                       | Greenish yellow | Violet     | Dark violet   |
| Powder+1% Iodine                                    | Greenish brown  | Purple     | Black         |
| Powder+Ethanol                                      | Brown           | Dark brown | Reddish brown |

Table 3: Phytochemical screening of *M. concanensis* flower extracts

| Name of the compound   | Name of the solvent (extracts) |               |                 |
|------------------------|--------------------------------|---------------|-----------------|
|                        | Chloroform                     | Diethyl ether | Petroleum ether |
| Alkaloids              | ++                             | -             | +               |
| Volatile oils          | ++                             | +             | +               |
| Fatty acids            | +                              | ++            | +               |
| Emodins                | +                              | ++            | -               |
| Flavonoids             | +++                            | ++            | ++              |
| Steroid tri terpenoids | ++                             | +             | +               |
| Anthracene glycosides  | +                              | ++            | +               |
| Phenolics              | -                              | +             | -               |
| Saponins               | +++                            | ++            | ++              |
| Tannins                | -                              | -             | -               |
| Carbohydrates          | ++                             | +             | -               |
| Cardiac glycosides     | +++                            | ++            | ++              |
| Reducing sugars        | ++                             | -             | -               |
| Amino acids            | ++                             | +++           | ++              |

+: Present in small concentration, ++: Present in moderately high concentration, +++: Present in very high concentration, -: Absent

number of phytochemicals (Table 3). Consequently, the chloroform extract was selected for the further free radical scavenging and cytotoxic investigations.

**Antibacterial activity of *M. concanensis* flower extracts:** The outcome of the antibacterial activity of *M. concanensis* flower extracts displayed the appreciable growth inhibition against the test pathogens (Table 4). The chloroform and petroleum ether extracts were displayed the maximum growth-inhibitory efficacy against the *E. coli* (18±0.6 mm). The *B. subtilis* exhibited a 15±0.3 mm inhibition zone in the chloroform extract. Otherwise, the *P. aeruginosa* displayed resistance to all the extract of *M. concanensis* flower.

**Minimum Bactericidal Concentration (MBC) of the *M. concanensis* flower extracts:** The growth inhibition effectiveness of chloroform, diethyl ether and petroleum ether extracts of *M. concanensis* flowers were inspected against the five human pathogenic bacterial strains. The strong antibacterial action of *M. concanensis* flowers was noted against the *V. cholerae* and *E. coli* in chloroform extract. The minimum inhibitory concentration was noted against the *V. cholerae* in chloroform extract, where the 190 µg mL<sup>-1</sup> of

chloroform extract was appreciably inhibited the growth of *V. cholerae*, subsequently, 220 µg mL<sup>-1</sup> of diethyl ether extract was notably suppressed the *B. subtilis* growth. The MBC values of chloroform, diethyl ether and petroleum ether extracts were depicted in Table 5.

**Antifungal activity of *M. concanensis* flower extracts:** As mentioned in Table 6, the *M. concanensis* flowers were displayed the appreciable antifungal potential against the tested pathogens. All three extracts were revealed the marked *in vitro* antifungal effects against the tested human pathogenic fungal strains. Among these strains, the *T. simi* and *T. mentagrophytes* (21±0.6 and 21±0.3 mm, respectively) were displayed the maximum sensitivity against the diethyl ether extract. The *A. flavus* exhibited 19±0.4 and 19±0.5 mm inhibition zones in the chloroform and diethyl ether extracts, respectively (Table 6). Whereas, the *T. simii* was found to be highly resistant to the petroleum ether extract. On the whole, all the extracts are markedly inhibited the growth of tested pathogenic fungal strains.

**Minimum Fungicidal Concentration (MFC) of *M. concanensis* flower extracts:** The outcomes of this investigation proved that the chloroform, diethyl ether and petroleum ether

Table 4: Antibacterial activity of crude extracts of *Moringa concanensis* flower

| Name of the organisms | Zone of the inhibition (mm) |               |                 |
|-----------------------|-----------------------------|---------------|-----------------|
|                       | Extracts                    |               |                 |
|                       | Chloroform                  | Diethyl ether | Petroleum ether |
| <i>E. coli</i>        | 18±0.6*                     | 15±0.8        | 18±0.6*         |
| <i>B. subtilis</i>    | 15±0.3*                     | 14±0.6        | 10±0.8          |
| <i>V. cholerae</i>    | 12±0.7                      | NZ            | 10±0.3          |
| <i>S. aureus</i>      | 11±0.9                      | 9±0.5         | NZ              |
| <i>P. aeruginosa</i>  | NZ                          | NZ            | NZ              |

Values are depicted as Mean±SD for the triplicate measurements, statistical significance was inspected via one-way ANOVA, subsequently, the DMRT test, where \*p<0.05, NZ: No zone formation

Table 5: MBC values (µg mL<sup>-1</sup>) of *M. concanensis* flower extracts

| Strains              | Chloroform extract (µg mL <sup>-1</sup> ) | Diethyl ether extract (µg mL <sup>-1</sup> ) | Petroleum ether extract (µg mL <sup>-1</sup> ) |
|----------------------|-------------------------------------------|----------------------------------------------|------------------------------------------------|
| <i>E. coli</i>       | 220±0.74*                                 | 350±0.65                                     | 236±0.68*                                      |
| <i>B. subtilis</i>   | 270±1.04                                  | 220±0.49*                                    | 310±1.32                                       |
| <i>V. cholerae</i>   | 190±0.82*                                 | >1000                                        | 540±0.49                                       |
| <i>S. aureus</i>     | 260±0.77*                                 | 420±0.72                                     | >1000                                          |
| <i>P. aeruginosa</i> | 639±0.81                                  | >1000                                        | >1000                                          |

Values are depicted as Mean±SD for the triplicate measurements, statistical significance was inspected via one-way ANOVA, subsequently, the DMRT test, where \*p<0.05

Table 6: Antifungal activity of *M. concanensis* flower extracts

| Name of the organisms    | Zone of inhibition (mm) |               |                 |
|--------------------------|-------------------------|---------------|-----------------|
|                          | Extracts                |               |                 |
|                          | Chloroform              | Diethyl ether | Petroleum ether |
| <i>A. flavus</i>         | 19±0.4*                 | 19±0.5*       | 15±0.4          |
| <i>C. albicans</i>       | 10±0.7                  | 16±0.4        | 10±0.7          |
| <i>T. simii</i>          | 13±0.6                  | 21±0.6*       | NZ              |
| <i>T. mentagrophytes</i> | 16±0.4*                 | 21±0.3*       | 15±0.5          |
| <i>T. rubrum</i>         | 13±0.3                  | 17±0.7        | 11±0.6          |

Values are depicted as Mean±SD for the triplicate measurements, statistical significance was inspected via one-way ANOVA, subsequently, the DMRT test, where \*p<0.05, NZ: No zone formation

Table 7: MFC values (µg mL<sup>-1</sup>) of *M. concanensis* flower extracts

| Strains                  | Chloroform extract (µg mL <sup>-1</sup> ) | Diethyl ether extract (µg mL <sup>-1</sup> ) | Petroleum ether extract (µg mL <sup>-1</sup> ) |
|--------------------------|-------------------------------------------|----------------------------------------------|------------------------------------------------|
| <i>A. flavus</i>         | 140±0.99                                  | 120±1.45*                                    | 180±0.78                                       |
| <i>C. albicans</i>       | 110±0.63*                                 | 280±0.33                                     | >1000                                          |
| <i>T. simi</i>           | 150±1.06                                  | 180±0.79                                     | 260±1.21                                       |
| <i>T. mentagrophytes</i> | 100±0.78*                                 | 190±1.36                                     | 140±0.66*                                      |
| <i>T. rubrum</i>         | 180±1.33                                  | 140±0.99                                     | 210±0.39                                       |

Values are depicted as Mean±SD for the triplicate measurements, statistical significance was inspected via one-way ANOVA subsequently, the DMRT test, where \*p<0.05

extracts of *M. concanensis* flower exhibited a remarkable antifungal potential against the tested pathogenic fungal strains. The strong antifungal outcome was noted against the *T. mentagrophytes* subsequently *C. albicans* in chloroform extract, where the 100 µg mL<sup>-1</sup> of chloroform extract was noticeably inhibited the growth of *T. mentagrophytes*, subsequently, 110 µg mL<sup>-1</sup> of chloroform extract was notably diminished the growth of *C. albicans*. The MFC values of chloroform, diethyl ether and petroleum ether extracts were displayed in Table 7.

#### Effect of *M. concanensis* flower extract against HepG2 cell

**line:** The result of the MTT cytotoxicity test proved that the chloroform extract of *M. concanensis* flowers displayed an appreciable cytotoxic potential against the human liver cancer (HepG2) cells. The treatment with the *M. concanensis* flower extract has drastically diminished the viability of human liver cancer (HepG2) cell lines in a dose-dependent mode. Consequently, it was proved that the chloroform extract of *M. concanensis* flowers were markedly diminished the viability of HepG2 cells. Figure 1 displaying the effect of chloroform

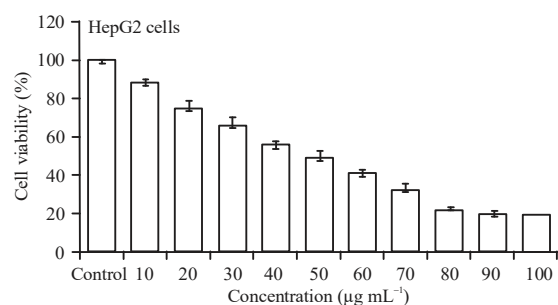


Fig. 1: Effect of chloroform extract of *M. concanensis* flower on HepG2 cell viability

extract of *M. concanensis* flowers HepG2 cell viability. Human liver cancer (HepG2) cells were cultured in DMEM with 10% fetal bovine serum. The cells were then treated with 10-100 µg mL<sup>-1</sup> of chloroform extract of *M. concanensis* flowers. The control and treated cells were subjected to MTT assay and the results were statistically investigated.

**Effect of *M. concanensis* flower extract against the HepG2 cell morphology:**

The chloroform extract of *M. concanensis* flowers was subjected to inspect the synergistic effect on the morphology of HepG2 cells. The morphological investigation

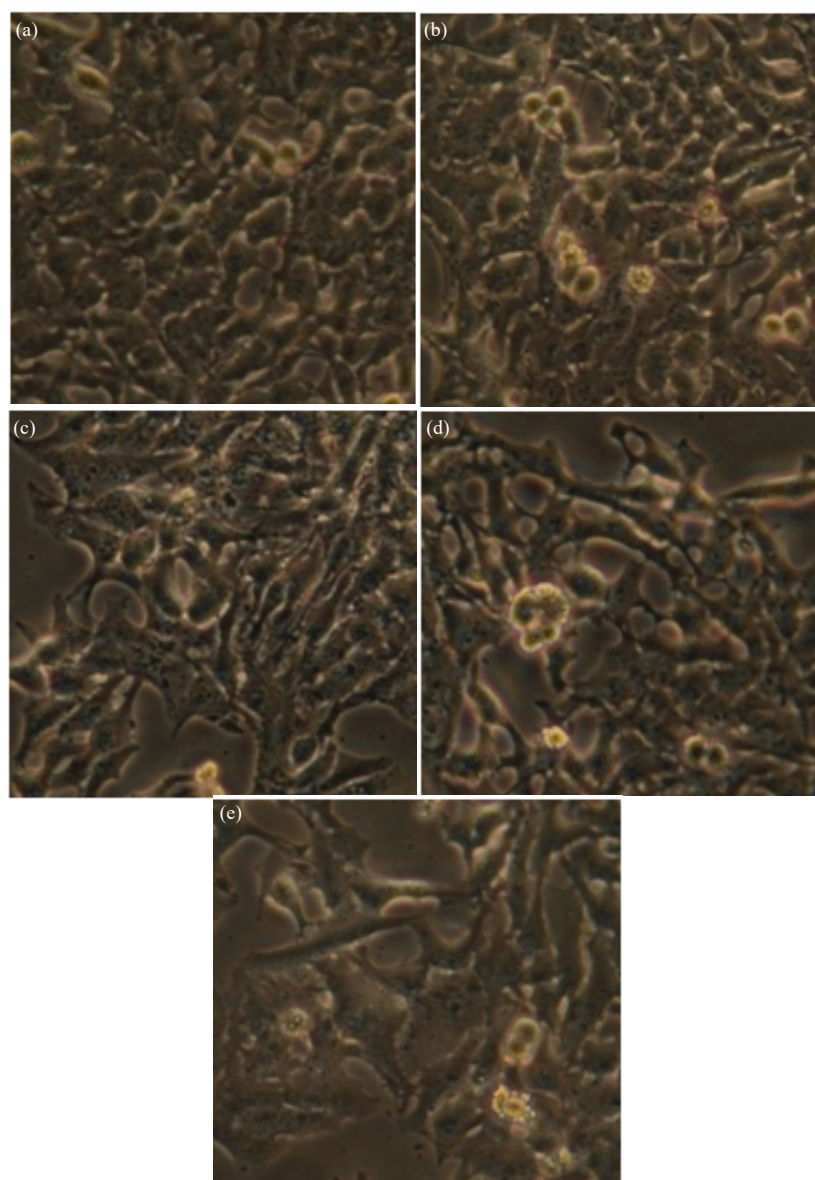


Fig. 2(a-e): Effect of chloroform extract of *M. concanensis* flower on HepG2 cells (a) Control, (b) 25, (c) 50, (d) 75, (e) 100 µg mL<sup>-1</sup>

of *M. concanensis* flower extract-supplemented HepG2 cells were displayed the drastic alterations on the morphology as well as the total amount of adherent cells. There are vast differences were exhibited on the morphology of extract treated HepG2 cells than the untreated control cells. The extract treatment ( $25\text{-}100\text{ }\mu\text{g mL}^{-1}$ ) displayed the tremendous morphological alterations that include unequal shape, rounding, cell shrinkages and detachments among cells on the HepG2 cells (Fig. 2a-e).

## DISCUSSION

The findings of this investigation was proved that the flowers of *M. concanensis* was appreciably inhibited the growth of pathogenic microorganisms as well as, it was displayed the potential cytotoxicity against the human liver cancer (HepG2) cells. In this decade, the emergence of multidrug-resistant microbes owing to the serious health consequences among populations worldwide, because they acquired the resistance because of recurrent and mishandling utilization of antibiotics. Consequently, the alternative approaches were needed urgently to counteract these issues with natural sourced novel agents<sup>22</sup>. The antimicrobial potential of *M. concanensis* flower extracts was inspected through the disc diffusion technique, MBC and MFC against the human-specific clinical pathogens. The outcomes of qualitative phytochemical screening revealed that the occurrence of flavonoids, alkaloids, volatile oils, cardiac glycosides, carbohydrates, amino acids and steroid triterpenes as the probable active constituents occurs in the *M. concanensis* flower extracts. The occurrence of these secondary metabolites in plants is accountable to generate the biological effects in humans as well as animals and it is accountable for their utilization as herbals<sup>23</sup>. These bioactive constituents are also accountable to protect the plants against the infection via microorganisms<sup>24,25</sup>.

The fluorescence is a phenomenon that is exhibited via reacting with different kinds of phytochemical constituents that occur in plant samples. A few phyto-compounds have displayed the fluorescence in the apparent daylight<sup>26</sup>. This investigation can assist the researchers to recognize and confirm the phytochemical status in the test plant material. These data may deed as a reference for accurate detection of exact plant specimen along with their phytochemical profile<sup>27</sup>. The *M. concanensis* flower extracts have possessed the noticeable antimicrobial and cytotoxic potential. The scientific investigation of plant specimens that have been utilized for conventional remedial purposes is essential to identify the potent antimicrobial agents. The *in vitro* antimicrobial

examination is a prime phase to the inspection of novel antimicrobial agents<sup>28</sup>. The global impact of infectious ailments is regarded with the treatment failures linked with multidrug-resistant pathogens and now it has become as a global concern to public health<sup>29,30</sup>. In this current exploration, the antimicrobial potential of flower extracts of *M. concanensis* was inspected against the different human pathogenic bacterial strains like *B. subtilis*, *S. aureus*, *E. coli*, *V. cholerae*, *P. aeruginosa* and the fungal strains were *A. flavus*, *C. albicans*, *T. simii*, *T. mentagrophytes* and *T. rubrum*. Among these bacterial species, *E. coli* has noted their more sensitivity in chloroform and petroleum ether extracts ( $18\pm0.6$  mm). Whereas, *P. aeruginosa* found to be highly resistant in all these extracts. The fungal species *T. simii* and *T. mentagrophytes* were found to be highly sensitive ( $21\pm0.6$  and  $21\pm0.3$  mm, respectively) in diethyl ether extract whereas, the *T. simii* was found to be highly resistant in petroleum ether extract.

The MBC of the *M. concanensis* flower extracts was noted in chloroform and petroleum ether extract against *V. cholerae*, the  $190\text{ }\mu\text{g mL}^{-1}$  of chloroform extract inhibited the visible growth, subsequently the *E. coli*, the  $220\text{ }\mu\text{g mL}^{-1}$  of chloroform extract of *M. concanensis* flower completely inhibited the visible growth of *E. coli* bacteria. The  $220\text{ }\mu\text{g mL}^{-1}$  of diethyl ether extract inhibited the visible growth of *B. subtilis* (Table 5). This outcome was proved that the chloroform extract of *M. concanensis* flower displayed the appreciable antibacterial effectiveness than the other extracts. The *P. aeruginosa* exhibited a maximum resistance to all the tested extracts. The potent antifungal effect was noted against the *T. mentagrophytes* subsequently *C. albicans* in chloroform extract, where the  $100\text{ }\mu\text{g mL}^{-1}$  of chloroform extract was appreciably inhibited the growth of *T. mentagrophytes*, subsequently,  $110\text{ }\mu\text{g mL}^{-1}$  of chloroform extract was notably diminished the growth of *C. albicans*. The MFC values of chloroform, diethyl ether and petroleum ether extracts were displayed in Table 7.

The cancer is a foremost ailment accountable for numerous death incidences worldwide<sup>31</sup>. The many cancer incidences were often diagnosed at the very late stage though the many cancer incidences were no longer treatable for remedial methods. Besides, the effectiveness of existing healing approaches for sarcoma was accountable for additional damages and declining the vast excitable pathways of drug metabolism and resistance to drugs linked to transporting proteins of malignant cells<sup>32</sup>. The cancer cell death has happened in numerous ways whenever the cells get

exposed to cytotoxic agents, like plant extracts and drugs. Normally, two kinds of the cell took place frequently that is apoptosis and necrosis. The apoptosis was undergone due to the many molecular and cellular mechanisms for example; contraction of cells, condensation of cells, fragmentation of DNA, blebbing of membranes as well as generation of apoptotic bodies<sup>33-36</sup>. In this current investigation, it was proved that the treatment of chloroform extract of *M. concanensis* flowers was noticeably suppressed the growth of the human liver cancer (HepG2) cells via altering the morphology of cells like cell shrinkages.

The results of the MTT cytotoxicity examination confirmed that the chloroform extract of *M. concanensis* flowers displayed an appreciable cytotoxic potential against the human liver cancer (HepG2) cell line. The treatment with the *M. concanensis* flower extracts has drastically diminished the viability of human liver cancer (HepG2) cell lines in a dose-dependent mode. The chloroform extract of *M. concanensis* flowers were also subjected to investigate morphology altering potential against HepG2 cells. The morphological investigation of *M. concanensis* flower extract-treated HepG2 cells was displayed the noticeable alterations in the morphology as well as the total amount of cells. The extract treatment exhibited incredible morphological alterations like unequal shape, rounding, cell shrinkages and detachments of HepG2 cells (Fig. 2a-e). From the findings, it was proved that the *M. concanensis* flowers were revealed the incredible antimicrobial potential against the human-specific pathogenic bacterial and fungal strains and displayed the appreciable cytotoxicity against the human liver cancer (HepG2) cell lines.

## CONCLUSION

The novel findings of this research investigation were evidence that the *M. concanensis* flowers were found to highly effective against the human-specific bacterial and fungal pathogenic strains. The findings were also proved that the *M. concanensis* flower extract treatment was diminished the cell viability of human liver cancer (HepG2) cell lines via stimulating the morphological alterations like rounding, cell shrinkages and detachments. Accordingly, it was concluded that the *M. concanensis* flower can be a promising source to identify the enhanced antimicrobial as well as cancer cytostatic agents. However, further research in the future was needed to elucidate the precise therapeutic mechanism of *M. concanensis* flower against the clinical pathogens.

## SIGNIFICANCE STATEMENT

This study was evidenced that the flowers of *Moringa concanensis* (Nimmo) has the immense phytochemicals and possess the potential antimicrobial activity against the infections causing pathogenic microbes. The findings of this study may disclose the pharmacological benefits of this plant and also help to the researchers to develop a novel antimicrobial compounds.

## REFERENCES

1. Vivas, R., A.A.T. Barbosa, S.S. Dolabela and S. Jain, 2019. Multidrug-resistant bacteria and alternative methods to control them: An overview. *Microb. Drug Resist.*, 25: 890-908.
2. Van Duin, D. and D.L. Paterson, 2016. Multidrug-resistant bacteria in the community: Trends and lessons learned. *Infect. Dis. Clin.*, 30: 377-390.
3. Whaley, S.G., E.L. Berkow, J.M. Rybak, A.T. Nishimoto, K.S. Barker and P.D. Rogers, 2017. Azole antifungal resistance in *Candida albicans* and emerging non-*albicans* *Candida* species. *Front. Microbiol.*, Vol. 7. 10.3389/fmicb.2016.02173.
4. Mostafa, A.A., A.A. Al-Askar, K.S. Almaary, T.M. Dawoud, E.N. Sholkamy and M.M. Bakri, 2018. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J. Biol. Sci.*, 25: 361-366.
5. Lazarou, R. and M. Heinrich, 2019. Herbal medicine: Who cares? The changing views on medicinal plants and their roles in British lifestyle. *Phytother. Res.*, 33: 2409-2420.
6. Mehlhorn, H., 2001. *Encyclopedic Reference of Parasitology*, Volume 1: Biology, Structure, Function. 2nd Edn., Springer, Berlin, Germany, ISBN-13: 9783540668190, Pages: 676.
7. Salmanzadeh, R., M. Eskandani, A. Mokhtarzadeh, S. Vandghanooni and R. Ilghami *et al.*, 2018. Propyl Gallate (PG) and tert-butylhydroquinone (TBHQ) may alter the potential anti-cancer behavior of probiotics. *Food Biosci.*, 24: 37-45.
8. Luo, D., K.A. Carter, D. Miranda and J.F. Lovell, 2017. Chemophototherapy: An emerging treatment option for solid tumors. *Adv. Sci.*, Vol. 4, No. 1. 10.1002/adv.201600106.
9. Anbazhakan, S., R. Dhandapani, P. Anandhakumar and S. Balu, 2007. Traditional medicinal knowledge on *Moringa concanensis* Nimmo of Perambalur district, Tamilnadu. *Ancient Sci. Life*, 26: 42-45.
10. Rani, A., N. Zahirah, K. Husain and E. Kumolosasi, 2018. *Moringa* genus: A review of phytochemistry and pharmacology. *Front. Pharmacol.*, Vol. 9. 10.3389/fphar.2018.00108.

11. Balakrishnan, B.B. and K. Krishnasamy, 2018. Evaluation of free radical screening and antioxidant potential of *Moringa concanensis* Nimmo-a medicinal plant used in Indian traditional medication system. Int. J. Pharm. Pharmaceut. Sci., 10: 91-97.
12. Joy, A.E., S.B. Kunhikatta and S. Manikkoth, 2013. Anti-convulsant activity of ethanolic extract of *Moringa concanensis* leaves in Swiss albino mice. Arch. Med. Health Sci., 1: 6-9.
13. Kale, S., G. Gajbhiye and N. Chaudhari, 2010. Formulation and *in-vitro* evaluation of *Moringa concanensis*, Nimmo. seed oils sunscreen cream. Int. J. PharmTech Res., 2: 2060-2062.
14. Kokoski, C.J., R.J. Kokoski and F.J. Slama, 1958. Fluorescence of powdered vegetable drugs under ultraviolet radiation. J. Am. Pharmaceut. Assoc., 47: 715-717.
15. Harborne, J.B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 1st Edn., Chapman and Hall, London, UK., ISBN: 978-94-009-5921-7, Pages: 271.
16. Kokate, C.K., 1997. Practical Pharmacognosy. 4th Edn., Vallabh Prakashan, New Delhi, India, pp: 108-111.
17. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.
18. Hofman, V.J., M.I. Ilie, C. Bonnetaud, E. Selva and E. Long *et al*, 2011. Cytopathologic detection of circulating tumor cells using the isolation by size of epithelial tumor cell method: Promises and pitfalls. Am. J. Clin. Pathol., 135: 146-156.
19. Wilkins, T.D., L.V. Holdeman, I.J. Abramson and W.E.C. Moore, 1972. Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother., 1: 451-459.
20. Curcic, M.G., M.S. Stankovic, I.D. Radojevic, O.D. Stefanovic and L.R. Comic *et al*, 2012. Biological effects, total phenolic content and flavonoid concentrations of fragrant yellow onion (*Allium flavum* L.). Med. Chem., 8: 46-51.
21. Hamdi, A., K. Majouli, G. Flamini, B. Marzouk, Z. Marzouk and Y.V. Heyden, 2017. Antioxidant and anticandidal activities of the Tunisian *Haplophyllum tuberculatum* (Forssk.) A. Juss. essential oils. S. Afr. J. Bot., 112: 210-214.
22. Omara, S.T., 2017. MIC and MBC of honey and gold nanoparticles against methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) coagulase-positive *S. aureus* isolated from contagious bovine clinical mastitis. J. Genet. Eng. Biotechnol., 15: 219-230.
23. Aumeeruddy, M.Z. and M.F. Mahomoodally, 2019. Combating breast cancer using combination therapy with 3 phytochemicals: Piperine, sulforaphane and thymoquinone. Cancer, 125: 1600-1611.
24. Ogbonnia, S.O., N.V. Enwuru, E.U. Onyemenem, G.A. Oyedele and C.A. Enwuru, 2008. Phytochemical evaluation and antibacterial profile of *Treulia africana* Decne bark extract on gastrointestinal bacterial pathogens. Afr. J. Biotechnol., 7: 1385-1389.
25. Ayaz, M., F. Ullah, A. Sadiq, F. Ullah, M. Ovais, J. Ahmed and H.P. Devkota, 2019. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. Chemico-Biol. Interact., 308: 294-303.
26. Rai, V., A. Kumar, V. Das and S. Ghosh, 2019. Evaluation of chemical constituents and *in vitro* antimicrobial, antioxidant and cytotoxicity potential of rhizome of *Astilbe rivularis* (Bodho-okhati), an indigenous medicinal plant from Eastern Himalayan region of India. BMC Complement. Altern. Med., Vol. 19. 10.1186/s12906-019-2621-6.
27. Bhandari, J., B. Muhammad, P. Thapa and B.G. Shrestha, 2017. Study of phytochemical, anti-microbial, anti-oxidant and anti-cancer properties of *Allium wallichii*. BMC Complement. Altern. Med., Vol. 17. 10.1186/s12906-017-1622-6.
28. Betoni, J.E.C., R.P. Mantovani, L.N., Barbosa, L.C. Di Stasi and A. Fernandes Jr., 2006. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem. Inst. Oswaldo Cruz, 101: 387-390.
29. Guschin, A., P. Ryzhikh, T. Rumyantseva, M. Gomberg and M. Unemo, 2015. Treatment efficacy, treatment failures and selection of macrolide resistance in patients with high load of *Mycoplasma genitalium* during treatment of male urethritis with josamycin. BMC Infect. Dis., Vol. 15. 10.1186/s12879-015-0781-7.
30. Martin, I., P. Sawatzky, G. Liu and M.R. Mulvey, 2015. Antimicrobial resistance to *Neisseria gonorrhoeae* in Canada: 2009-2013. Can. Commun. Dis. Rep., 41: 35-41.
31. Maucourt-Boulch, D., C. de Martel, S. Franceschi and M. Plummer, 2018. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. Int. J. Cancer, 142: 2471-2477.
32. Shi, L., Y.X. Wu, J.H. Yu, X. Chen, X.J. Luo and Y.R. Yin, 2017. Research of the relationship between  $\beta$ -catenin and c-myc-mediated Wnt pathway and laterally spreading tumors occurrence. Eur. Rev. Med. Pharmacol. Sci., 21: 252-257.
33. Shigematsu, H., K. Yoshida, Y. Sanada, S. Osada and T. Takahashi *et al*, 2010. Rapamycin enhances chemotherapy-induced cytotoxicity by inhibiting the expressions of TS and ERK in gastric cancer cells. Int. J. Cancer, 126: 2716-2725.
34. Dey, D., S. Das, H.R. Yadav, A. Ranjani and L. Gyathri *et al*, 2016. Design of a mononuclear copper(II)-phenanthroline complex: Catechol oxidation, DNA cleavage and antitumor properties. Polyhedron, 106: 106-114.
35. Saha, S.K., S. Sikdar, A. Mukherjee, K. Bhadra, N. Boujedaini and A.R. Khuda-Bukhsh, 2013. Ethanolic extract of the Goldenseal, *Hydrastis canadensis*, has demonstrable chemopreventive effects on HeLa cells *in vitro*: Drug-DNA interaction with calf thymus DNA as target. Environ. Toxicol. Pharmacol., 36: 202-214.
36. Ansil, P.N., P.J. Wills, R. Varun and M.S. Latha, 2014. Cytotoxic and apoptotic activities of *Amorphophallus campanulatus* (Roxb.) Bl. tuber extracts against human colon carcinoma cell line HCT-15. Saudi J. Biol. Sci., 21: 524-531.