Screening of Emblica officinalis, Terminalia chebula & Terminalia bellarica for their Antioxidant, Antibacterial and Anti-Cytotoxic Activities

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Abstract

Natural products, in the field of drug discovery, remain a viable source of scaffolds with a wide range of structural diversity and bioactivities that can be developed directly or utilized as starting points for the development of novel medications. Because plant-based medications are natural, they pose no significant risks. Embolic officinal is (Alma) is a popular Ayurveda plant with a reputation for being a powerful revitalizing herb with the greatest vitamin C concentration. Embolic officinal is, terminally chebula & Terminally Billerica extracts have significant antibacterial properties against a variety of bacterial infections. They could also act as a potent antioxidant, antimicrobial and anti-cytotoxic agent, to combat various associated diseases. This work is therefore, focussed to screen and determine the ant oxidative, antimicrobial and anti-cytotoxic potential of Embolic officinal is, terminally chebula and Terminally Billerica.

Keywords: Natural products, *Emblica officinalis, Terminalia chebula, Terminalia bellarica*, antioxidative, antimicrobial, anti-cytotoxic

Introduction

Plant-derived medications are still a valuable resource in the fight against serious diseases, especially in developing nations. For the treatment of common ailments, approximately 60–80 percent of the world's population still uses traditional medicines[1]. The study of therapeutic plants has recently regained popularity. The main reason is that alternative medical systems, while functional, have a slew of side effects that frequently result in significant consequences. Because plant-based medication is natural, it poses no significant risks[2].

Emblica officinalis (amla) is a popular Ayurvedic plant with a reputation for being a powerful revitalizing herb with the greatest vitamin C concentration[3]. Amla, *Terminalia Chebula* & *Terminalia bellarica* extracts have significant antibacterial properties against a variety of bacterial infections. Antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, immunomodulatory, hypolipidemic, memory boosting, anticancer, antidiabetic, antidepressant, anti-ulcerogenic, insecticidal, larvicidal, and wound healing properties are also found in amla, phytochemicals. All these well-established activities can be used to treat a wide range of ailments in both human and animal patients. Because of its greater efficacy and lack of side effects, it can either replace traditional therapeutic agents or operate as an adjuvant therapeutic agent, boosting the total efficacy of conventional medicines[4]. All these well-established activities can be used to treat a wide range of ailments in both human and animal patients. Because of its greater efficacy and lack of side effects, it can either replace traditional therapeutic agents or operate as an adjuvant therapeutic agent, boosting the total efficacy of conventional medicines. Because of the low-cost factor and the improved antibacterial efficacy of the nanoparticles produced, biogenic production of nanoparticles from E. officinalis is gaining favour.

Emblica officinalis (amla), *Terminalia Chebula* & *Terminalia Bellarica* could act as a potent antioxidant, antimicrobial and anti-cytotoxic agent, to combat various associated diseases. There are non-plant-based medications that are extensively used in modern medicines to combat associated diseases; however, they leave behind, many serious complications and their prognosis is poor. Such complications are mostly observed in cancer chemotherapy. Amla increased the anti-cancer effects of two chemotherapeutic medicines, Mitomycin C and cisplatin, in a preliminary 2017 cell study[5]. It also shielded healthy cells from the medicines' genotoxic (DNA-damaging) effects. Rats given amla fruit pulp for seven days before receiving a lethal dose of radiation boosted their survival rate from 0% to 87.5% in 2007 animal research[6]. Natural products (NPs) have historically been a rich source of antimicrobial agents with a wide diversity of structures and biological activity[7]. In the field of drug discovery, NPs remain a viable source of scaffolds with a wide range of structural diversity and bioactivities that can be developed directly or utilized as starting points for the development of novel medications. NPs, on the other hand, provide technological difficulties to drug development, such as technical barriers to screening, isolation, characterization, and optimization, which contributed to a drop in the pharmaceutical industry's pursuit of natural products from the 1990s forward[8].

The Food and Drug Administration (FDA) regulates biological products, which are used to diagnose, prevent, treat, and cure diseases and medical problems. Biological goods are a broad category of products that often consist of huge, complicated molecules. These products are typically more difficult to describe than small molecule medications since they are created by biotechnology in a living system, such as a microbe, plant cell, or animal cell. Therapeutic proteins (such as filgrastim), monoclonal antibodies (such as adalimumab), and vaccinations are among the biological products licensed for use in the United States (such as those for influenza and tetanus)[9]. However, using biological products such as monoclonal antibodies in therapeutic approaches has various drawbacks, including the fact that it is a time-consuming process that can take anywhere from 6 to 9 months. They are very expensive and require a lot of effort to create. Antigens made up of small peptides and fragments may not be suitable because monoclonal antibodies may fail to detect the original antigen[10].

In these regard, plant-based products like amla may be a boon to counteract the disadvantages of the other mentioned approaches to treat various illnesses, and that comes with more benefits and less risks.

The Amla, *Terminalia Chebula* & *Terminalia bellarica* extract significantly and dose-dependently protected RBCs and plasma protein from ROS-induced stress, according to a study. Amla's protective impact on RBC could be attributed to its ability to reduce lipid peroxidation and maintain intracellular antioxidants. The antioxidant properties of amla may be due to its constituent phenolics and flavonoids, which inhibit lipid peroxidation, protein carbonylation, and plasma protein breakdown[11]. Previously, it was discovered that E. officinalis has a greater amount of ascorbic acid (1.28 % w/w), which is primarily responsible for its antioxidant activity. Embricanins A and B have almost 7.86 and 11.20 times the DPPH scavenging activity of vitamin C, respectively, while gallic acid and ellagic acid have around 6.25 and 3 times the activity of ascorbic acid, respectively[12]. Reddy *et al*. and Andican *et al.* studied the protective effects of *E. officinalis* fruit extract against alcohol-induced oxidative stress and discovered that polyphenols such flavonoids, tannins, and other components like ascorbic acid play a role in oxidative stress mitigation. These polyphenols are mostly found in *E. officinalis* fruit extract, and they are important for protecting against alcohol-induced oxidative stress by scavenging NO, which acts both as an antioxidant and a pro-oxidant[13,14].

Because of its potent medicinal values, amla extracts have antimicrobial properties such as antifungal, antiviral, and antibacterial activities. As a result, this plant is being investigated for developing novel and alternative antibacterial and complementary treatment options in biomedical science[4].

As a result, this work focussed to screen and determine the antioxidative, antimicrobial and cytotoxic potential of *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellarica.*

Materials and Methods

Cell Viability Assay: Spleen from BALB/c mice was extracted and transferred immediately to Dulbecco's Phosphate Buffer Saline (PBS) to remove any leftover connective tissue, adipose tissue, or other tissue, after which it was again transferred to another sterile petridish containing Modified Eagles Medium (MEM) without serum. The spleen was moved to a fresh petridish containing MEM and serum, and the cells were sliced into extremely small pieces using scissors to ensure that the spleenocytes are dispersed into the medium. The cell suspension was then transferred to a sterile centrifuge tube and allowed to settle for 10-15 minutes. After ascertaining that all tissue remnants are sedimented, the supernatant was carefully transferred to a fresh culture flask. The cells were observed for growth for next one week and the % viability was measured using the formula:

IC50 is calculated as follows: ((Control – Sample)/Control) *100

MTT Assay: 100 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in 20ml of PBS (5 mg/mL) and the prepared MTT solution was stored at 4°C (stable for at least 6 months). A subconfluent monolayer culture of the harvested primary spleenocytes was trypsinized and the cells were collected in growth medium containing serum (HAM's F12K media + 10% FBS).

The cell suspension was transferred to a 96-well plate, adding 100µl of the suspension into each well. The plate was incubated for the next 24h at 37^0 C to form a cell monolayer in each well. (at least for two population doubling). A stock solution of 1000 µg /ml was prepared in a solvent i.e., DMSO. And the further two-fold serial dilution was prepared in the growth medium to give 5 drug dilutions to treat against the cells. After the incubation the media from the well was carefully aspirated and 100µl of the drug (conc. 1000µl, 500µl, 250µl, 125µl, 62.5µl and 31.25µl) were seeded into the wells. The plate was again incubated for next 24h at 37^0 C. On the next subsequent day, the media with drug was removed from wells and 50µl of MTT was filled in each well of the 96-well plate. The plate was then incubated at 37^0 C for the next 3. The MTT was then removed from the wells without disrupting the produced formazan crystals and each well was treated with 50µl of iso-propyl alcohol to dissolve the formazan crystals incubating the plate for 30 minutes at room temperature in the dark. The absorbance was measured at 540nm in the ELISA Plate reader (Biotek Instruments Inc, USA). The findings were obtained by fitting non–linear regression curves and IC50 is calculated as follows: ((Control – Sample)/Control) *100

DPPH Free Radical Scavenging Assay (DPPH FRSA): To prepare the diphenyl picryl hydrazyl (DPPH) solution, 0.024g of DPPH was transferred to a volumetric flask and 50ml of absolute ethanol was added to it and dissolved, and the volume was made upto 100ml. The assay was carried out in a 96-well microtiter plate. To 200µl of the DPPH solution, 10µl of the standard (ascorbic acid) solution and the test samples were added separately in wells of the microtiter plate. The final concentration of the test and standard solutions used are 1000 to 1.95 µg/ml. The plates were incubated at 37°C for 20 minutes and their absorbance was measured at 490 nm, using ELISA reader. Radical scavenging activity was determined using the formula:

Scavenging activity (%) = (A0 - A1) / A0 * 100

Where, A0 represents the absorbance of the blank (no extract) and
A1 represents the absorbance of the extract or standard.

Cup Plate Method: Antimicrobial activity of extract of raw against various strains of bacteria (*E. coli* & *Bacillus*) were carried out by cup plate method. Each test organism was inoculated on nutrient broth (NB) and Sabouraud's dextrose broth (SDB) and incubated for 24h and 48h respectively. Sterile plates were then inoculated with the test organisms by spread plate method. They were bored using sterile metal borer. To the bores 100 µl of different concentration of extracts were added (1000 mg and 500 mg) incubated at 37ºC for 24 hours (nutrient agar) and 26ºC for 48h (Sabouraud's dextrose agar).

Results and Discussion

Cell Viability Assay:

Activity of all the plant extracts i.e., *Emblica officinalis* (Figure 1a), (*Terminalia chebula*) (Figure 1b) and (*Terminalia bellarica*) (Figure1c) were present in the cultured spleenocytes.

MTT Assay:

Among all the extracts, IC_{50} value was found the lowest in the extracts of *Terminalia bellarica* (Figure 2c), followed by *Emblica officinalis* (Figure 2a) *and Terminalia chebula* (Figure 2b)**,** suggesting that *Terminalia bellarica* is more effective in lower concentrations when compared to the other two extracts.

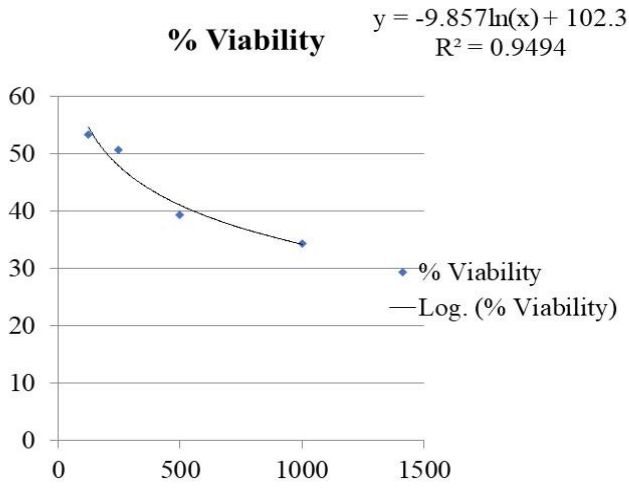
DPPH Free Radical Scavenging Assay (DPPH FRSA):

IC_{50} value was found the lowest in the extracts of *Terminalia chebula* (Figure 2b), followed by *Terminalia bellarica* (Figure 2c) and *Emblica officinalis* (Figure 2a), suggesting that the extracts of *Terminalia chebula* is required in lesser concentration than the other two extracts, in order to scavenge the free radicals, which further indicates that presence of antioxidant molecules is more in *Terminalia chebula* than in the extracts of *Emblica officinalis* and *Terminalia bellarica*.

Cup Plate Method:

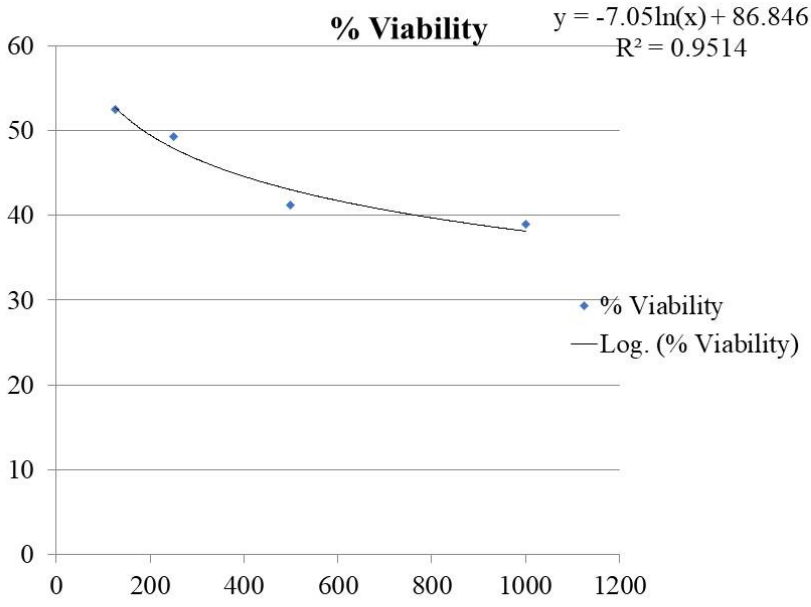
It was observed that *E. coli & Bacillus* colony was absent in the extract of *Emblica officinalis* (Figure 4a & 4b) at all given concentrations, but was present in the extracts of *Terminalia chebula* (Figure 4c & 4d) and *Terminalia bellarica* (Figure 4e and 4f) in minute amounts. This is suggestive of the fact that the antimicrobial activity is higher in *Emblica officinalis* than in *Terminalia chebula* and *Terminalia bellarica*, and this strong antimicrobial activity of *Emblica officinalis* may be attributed to its phytochemical constituents that prevents microbial growth.

Figure 1a: % Viability of *Emblica officinalis* in Spleenocytes



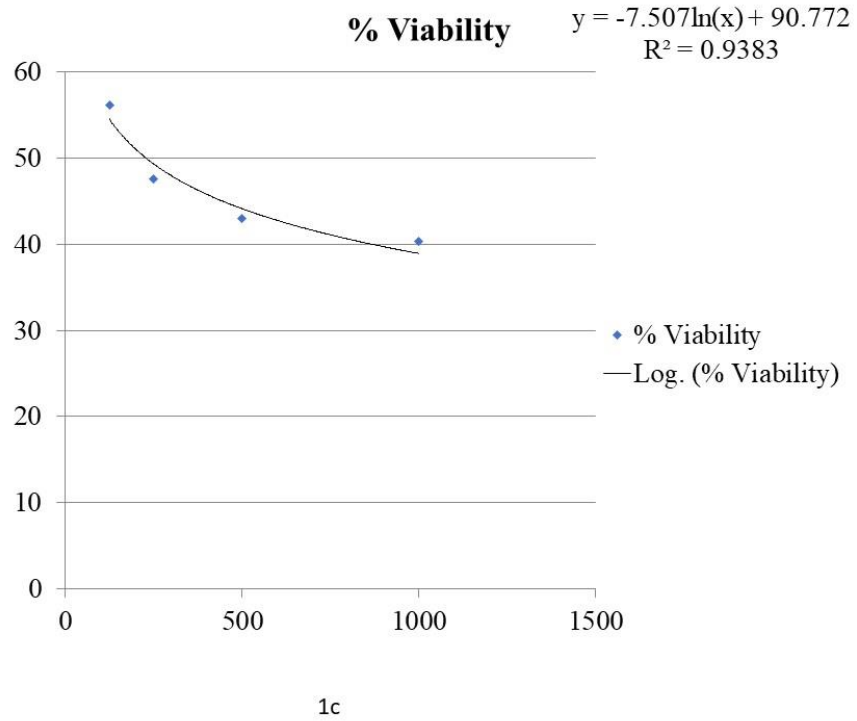
1a

Figure 1b: % Viability of *Terminalia chebula* in Spleenocytes



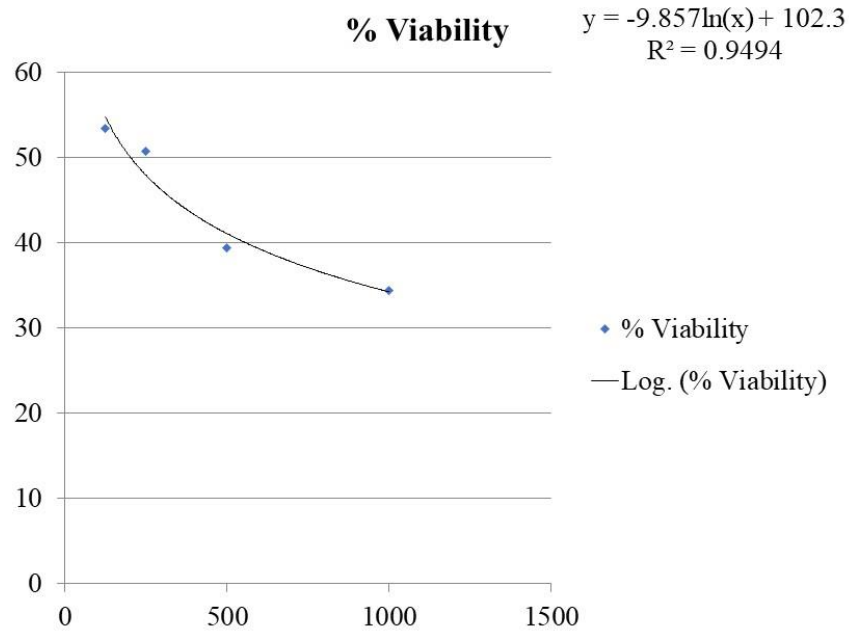
1b

Figure 1c: % Viability of *Terminalia bellarica* in Spleenocytes



1c

Figure 2a: IC_{50} of *Emblica officinalis* in Spleenocytes by MTT Assay



2a

Figure 2b: IC_{50} of *Terminalia chebula* in Spleenocytes by MTT Assay

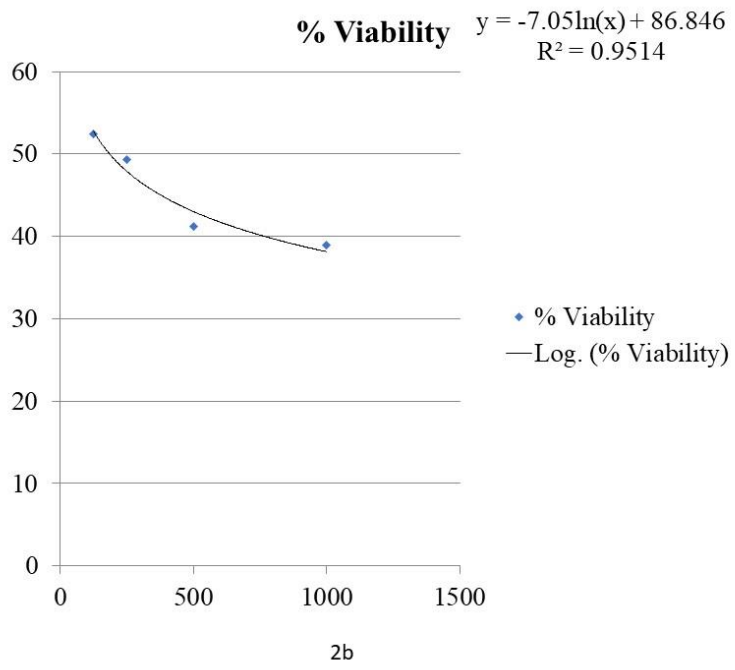


Figure 2c: IC_{50} of *Terminalia bellarica* in Spleenocytes by MTT Assay

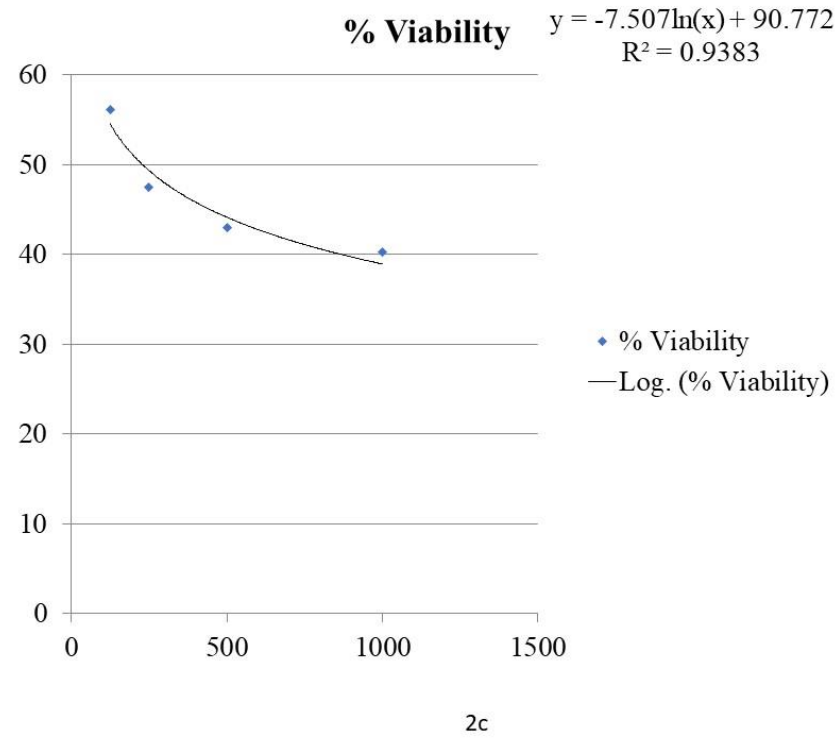
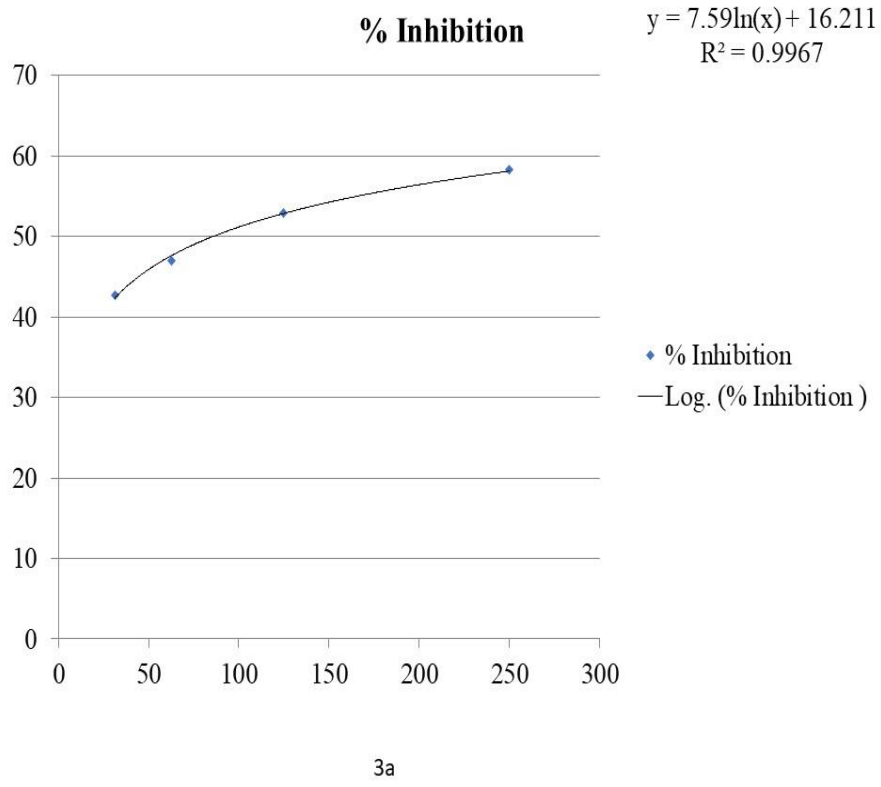
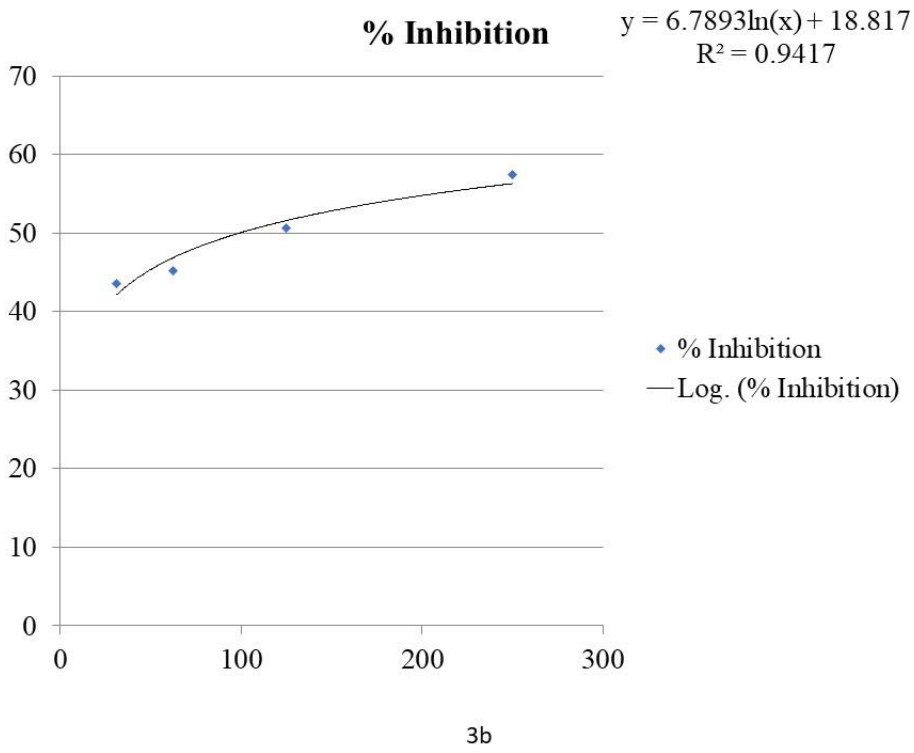


Figure 3a: IC_{50} of *Emblica officinalis* in Spleenocytes by DPPH FRSA



3a

Figure 3b: IC_{50} of *Terminalia chebula* in Spleenocytes by DPPH FRSA



3b

Figure 3c: IC_{50} of *Terminalia bellarica* in Spleenocytes by DPPH FRSA

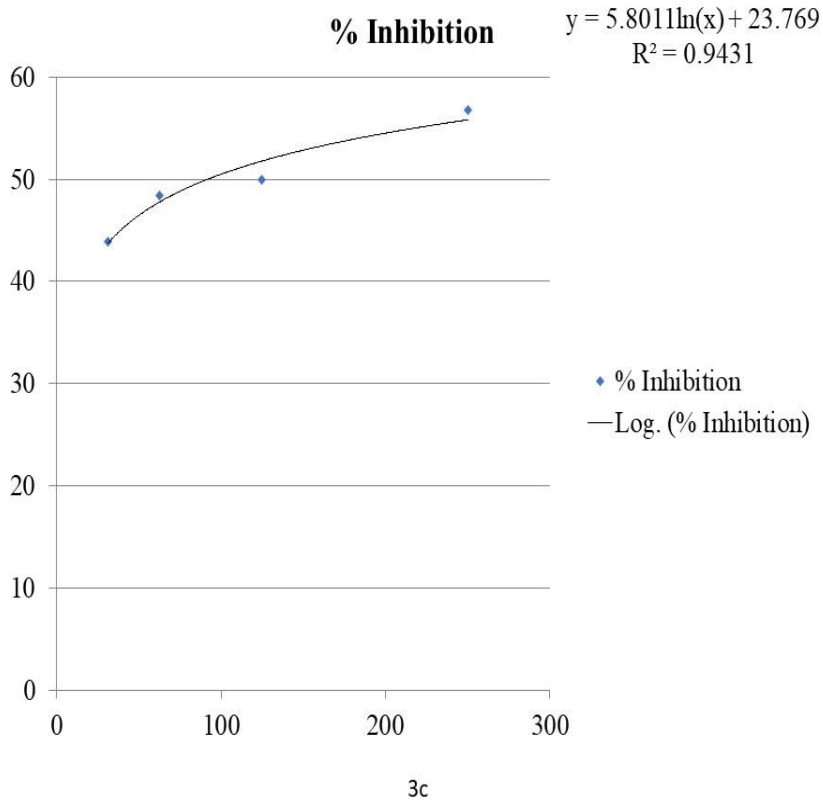


Figure 4a: Antimicrobial activity of *Emblica officinalis* extracts against *E. coli*

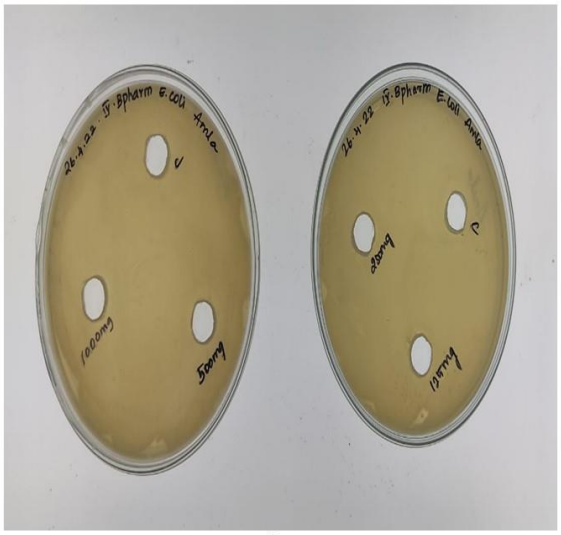
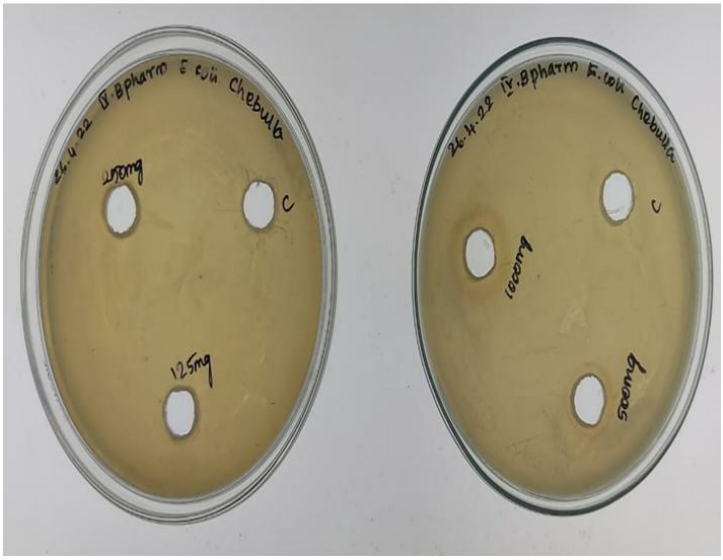


Figure 4b: Antimicrobial activity of *Emblica officinalis* extracts against *Bacil*

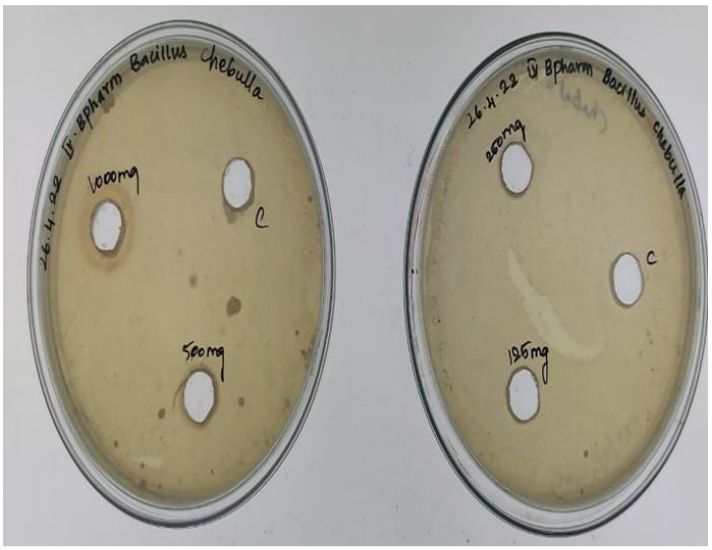
4b

Figure 4c: Antimicrobial activity of *Terminalia chebula* extracts against *E. coli*



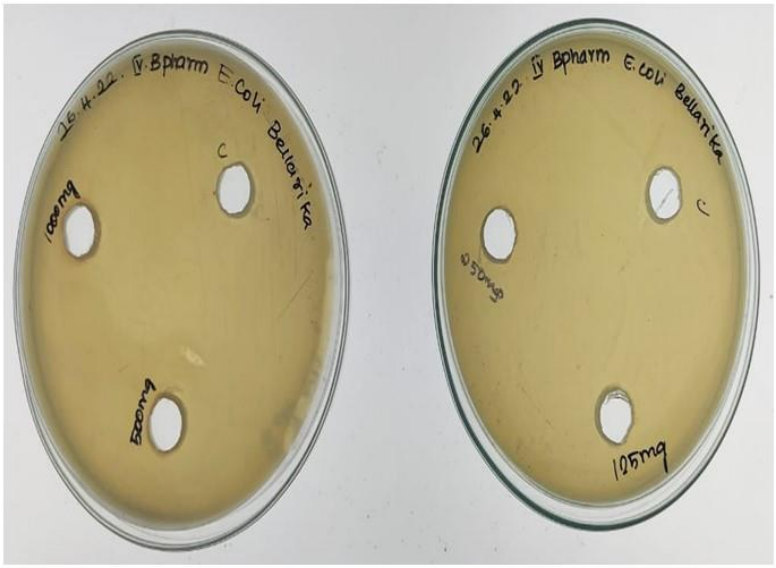
4c

Figure 4d: Antimicrobial activity of *Terminalia chebula* extracts against *Bacillus*



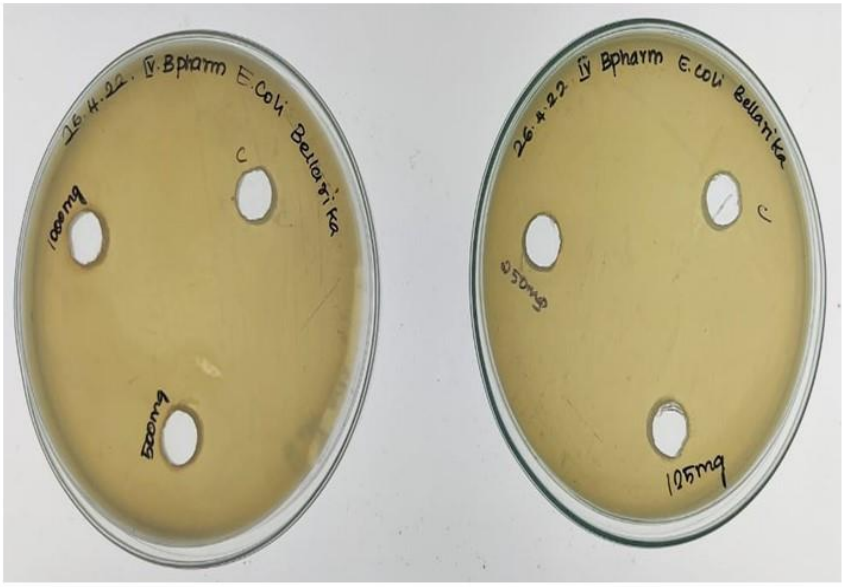
4d

Figure 4e: Antimicrobial activity of *Terminalia bellarica* extracts against *E. coli*



4e

Figure 4f: Antimicrobial activity of *Terminalia bellarica* extracts against *Bacillus*



4f

Conclusion

Based on our findings, we conclude that the extracts of *Emblica officinalis*, *Terminalia chebula* and *Terminalia bellarica* were able to scavenge free radicals, exhibited cytoprotective & antimicrobial activity effectively. Hence, these plants may be utilized as potent antioxidant, cytoprotective and antimicrobial molecules, and may be further considered in drug discovery & development against various diseases.

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