IJPSR (2021), Volume 12, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 October 2020; received in revised form, 10 March 2021; accepted, 24 May 2021; published 01 October 2021

PHARMACOGNOSTICAL EVALUATION OF *TERMINALIA CHEBULA* FRUIT EXTRACT AGAINST RESPIRATORY TRACT INFECTIOUS PATHOGENS

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Keywords:

Biocompatibility, Cell viability, Cytotoxicity, Lung cell line, Time Kill assay

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ABSTRACT: To evaluate the antibacterial activity of methanolic extract of *Terminalia chebula*, commonly called Haritaki was tested against respiratory tract infection-causing bacteria. The antibacterial activities were assessed by agar well diffusion, broth dilution and time-kill methods. It showed the best antibacterial activity against *Klebsiella pneumonia*e among the tested respiratory infection-causing bacteria (*Streptococcus pneumonia* and *Streptococcus pyogenes*) in the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), at 100 mg/ml, respectively. Killing ability depends on the time and concentration of the extract, which was found optimum at 20 h at 100 mg/ml. The cell viability and cytotoxicity of the extract were tested on swine Lung Cell lines with different concentrations (100-500 mg/ml). The viability of the cells was more at 100 mg/ml, whereas the viability gradually decreases by increasing the concentration was determined by 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. The extract showed minimal hemolytic effect in human red blood cells in Biocompatibility assay at 100 mg/ml, which was tested against standard drug levo flaxacin. It suggests that the methanolic extract of *T. chebula*may be effectively used against respiratory tract infection-causing bacteria and could be a better alternative for an existing antibiotic.

INTRODUCTION: Infectious diseases pose grave threats to health and human Survival [1]. According to WHO 2012, morbidity and mortality due to infectious diseases such as diarrhea, malaria, respiratory diseases, tuberculosis are considered the big challenge for developing countries than developed one. Apart from the health of an individual, it poses adverse effect on whole societies and economy [2].

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DOI: 10.13040/IJPSR.0975-8232.12(10).5446-57

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(10).5446-57

India has 18% of the worldwide population and an increasing rate of respiratory ailments. Of the total global disability-adjusted life years (DALYs) due to chronic respiratory diseases in 2016, 32.0% occurred in India, especially chronic obstructive pulmonary disease (COPD) and asthma were responsible for 75.6 and 20% of chronic respiratory disease (DALYs), respectively [3].

Bacteria are known to cause primary infection or superinfection namely, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Moraxella catarrhalis* [4]. Regardless of the presence of strong antibiotics, multi-resistant strains are consistently showing up, forcing the requirement for a changeless inquiry and improvement of new medications. Anti-

microbial abuse, nearness of fake or low-quality anti-infection agents, unacceptable cleanliness and helpless day-to-day environments are the major main thrusts behind the rise and spread of anti-toxin obstruction, particularly in the developing nations where the burden of these contaminations is high [5, 6]. So, the nation is in need of searching alternative medicine to combat the resistance. For Centuries plants have been used throughout the world as drugs and remedies for various diseases also promising alternative treatment option [7].

Terminalia chebula, commonly called Haritaki a therapeutic plant that belongs to genus Terminalia (family- Combretaceae), is developed in Tibet, Taiwan, China and India [8]. It has been widely used for the treatment of Upper and lower respiratory tract infection, fever including piles, diarrhea, gout, heart and bladder diseases [9]. It has been reported haritaki has strong antioxidant properties and active against gram-positive and negative bacteria [10]. The observed health benefits may be credited to the presence of phytochemicals such as gallic acid, chebulagic acid, corilagin, mannitol, ascorbic acid (vitamin C) and other compounds [11].

Apart from phytoconstituents, the presence of polysaccharides exhibits a large range of pharmacological effects [12]. Six phenolic compounds were isolated and identified as gallic acid, punicalagin, iso terchebulin, 1,3,6-tri-O-galloyl-β-D-glucopyranose, chebulagic acid and chebulinic acid showed stronger 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and melanin inhibitory activities [13]. Extracts of *T. bellerica* and *T. chebula* have been reported to possess in vitro αglucosidase in hi bitiory activities and anti-diabetic actions in animal models [14]. In this connection, this study focused on evaluating the antibacterial, antioxidant and cytotoxicity activities of the methanolic extract of *T. chebula* against respiratory infection-causing bacteria in relation to their ethnobotanical uses.

MATERIALS & METHODS: Materials and Sources: *T. chebula* dried fruits were collected from the local Ayurvedic Store, at Coimbatore and it was authenticated at Botanical Survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu. A voucher specimen was preserved in our laboratory for future reference. From the dried fruits, seeds from the individual fruits were removed, and dried fruit pulp was crushed into a fine powder using grinder. The powder was stored in a closed vessel for future use.

Methanolic Extraction of Haritaki: Ten grams of sample powder were weighed and dissolved in 100 ml of methanol (1:10 ratio). Totally three aliquots were prepared to perform various extraction methods. The first flask was placed in a shaker 120 rpm for 24 h **Fig. 1A** 15; another flask was placed in a microwave oven, the frequency between 300 MHz to 300 GHz for 5 min **Fig. 1B** [16]. The third flask kept into ultrasonic processor (Bandi Technologies, New Delhi) employed for ultrasonic extraction at (20kHz) for 3 h **Fig. 1C** [15]. The extract was evaporated to dryness under reduced pressure by a rotary evaporator at 35 °C. The dried methanolic extract was freeze-dried and stored in airtight container for future experimental purposes. Sonicator extraction more preferable for the entire work than the other two extractions based on the anti-biotic screening results.

A. SHAKER **B. MICROWAVE OVEN** **C. ULTRASONICATOR**

FIG. 1: METHANOLIC EXTRACTION OF *T. CHEBULA*

Sterility Test: Sterility of the extract was confirmed by pour plate technique. 100 µl of the extract (0.1g in 100 µl methanol) was mixed with 20 ml of nutrient agar medium and poured in sterile petriplates. Plates were kept in incubation at 370 ℃ for 24 h. After incubation plates were viewed for any contamination [13].

Phytochemical Analysis: The preliminary phyto-chemical studies were performed to test the different chemical groups present in the drug. 10% (w/v) solution of extract was taken in the respective tubes for an individual test. Tests for Tannin, Cardio glycoside, Ant hroquinine, Phenol, Flavanoid, Saponin, Alkaloid, Terpenoid, Steroid, Glycoside tests were performed based on standard procedure of [14].

Characterization Study:

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy (Shimadzu, Japan), an analytical technique used to observe chemical properties of test samples. FTIR spectral analysis was carried out through potassium bromide (KBr) pellet method in 1:30 ratios (NPs: kBr) and the spectrum recorded at the resolution of 4 cm^{-1} and the wavelength range from 500-4500 cm^{-1}. The compounds were confirmed by comparing with reference data from available literature [17].

Antioxidant Activity: The total antioxidant property of the methanolic extract of *T. chebula* was measured using a spectrophotometer method at 100-500 mg/ml. Ascorbic acid is used as a standard. The experiment was conducted in triplicate, and the values are expressed as µg equivalents of ascorbic acid per µg/mL of extract [18].

Ferric Ion Reducing Power Assay: The chelating effect on ferrous ions of the prepared extracts was estimated by the method of 18 with slight modifications. 100 µl of the test sample (1 mg/mL) was taken and make upto 3 ml with methanol. A volume of 740 µl of methanol was added to 20 µl of 2 mM $FeCl_2$. The reaction was initiated by the addition of 40 µl of 5 mm Mferrozine into the mixture, which was then left at room temperature for 10 min and then the absorbance of the mixture was determined at 562 nm.

DPPH Radical Scavenging Assay: The anti-oxidant capacity of the fruit extract was confirmed by the DPPH scavenging assay according to18 with slight modifications. Different concentrations of the extracts and the standard were mixed with equal volume of methanol. Then 50 µl of DPPH solution (1 mM) was pipetted into the previous mixture and stirred thoroughly. The resulting solution was kept standing for 2 min before the optical density (OD) was measured at $\lambda = 517$ nm.

Hydroxy Radical Scavenging Activity: The hydroxyl radical scavenging activity of *T. chebula* was performed. The reaction mixture contained deoxyribose (2.8 mM), KH2PO4-NaOH buffer, pH 7.4 (0.05 M), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H2O2 (1 mM) and different concentrations of *T. chebula* extracts in a final volume of 2 ml.

The mixture was incubated at 37 ℃ for 30 min followed by the addition of 2 ml of TCA (2.8% w/v) and thiobarbituric acid. Thereafter it was kept for 30 min in a boiling water bath and cooled. The absorbance was recorded at 532 nm in a UV–VIS spectrophotometer.

Microorganisms Used: *Streptococcus pneumonia*, *Streptococcus pyogenes,* and *Klebsiella pneumoniae* (Ref No:46218) cultures were collected from the Bioline Laboratory, R.S Puram, Coimbatore, Tamil Nadu and stored at 40 ℃.

Antibacterial Activity: Antibacterial activity of methanolic extract of *T. chebula* against *S. pneumoniae, S. pyogenes* and *K. pneumoniae* strains were studied using Nutrient Agar and Muller Hilton Agar. MHA is a specific medium that was used to perform antibacterial activity. Several trials were taken to standardize the concentration of the extract which acts against pathogens.

Agar well Diffusion Method: Above mentioned Bacterial cultures were pre-lawned in each plate containing MHA medium. The Well was made by sterile cork borer and filled with different concentrations (100-500 mg/ml) of the extract and kept for incubation at 370 ℃ for 24 h. The diameter of each zone was noted and measured [11]. The bacteria which have minimum resistance against the treated compound will be taken for further study (Standard - Penicillin; Positive Control – Levo floxacin)

Determination of Minimum Inhibitory Concentration (MIC): The MIC of *T. chebula* against *K. pneumoniae* was determined by Standard method [14]. The test bacterial culture was grown in Mueller–Hinton broth (MHB) at 37 °C overnight and the Culture media (200 µl) was used as control.

100 µl of various concentrations of methanolic extract (100-500 mg/ml) were inoculated along with overnight grown bacterial culture in a polypropylene 96-well micro plate. Micro plates were covered with parafilm and incubated properly for 24 h. After incubation, the absorbance was measured at 550 nm.

Determination of Minimum Bactericidal Concentration (MBC): MBC is the lowest concentration of antimicrobial agent that will not allow the growth of microbes into the antibiotic free media[12]. 10 µl of each aliquot from the MIC tubes were spreaded over the MHA petriplates and incubated at 370 °C for 24 h and examined for bacterial growth and the results were recorded.

Time Kill Assay: Time-kill assay of methanolic extract of *T. chebula* was carried out following the procedure described by [19]. The extracts were prepared as the concentration of MIC (100 -500 mg/ml). *K. pneumoniae* (10 µl) was added in the medium and incubated at 370 °C then add at desired concentrations of extract to relative wells accordingly and kept in incubation for next 24 h.

The titre plate was subjected for OD at 570 n mat time intervals of 0, 4, 8, 12 and 24h. Simul-taneously, the tests were performed with the reference anti-biotic levoflaxacin.

Collection of Swine Lung: Swine are similar to humans in anatomy, physiology and immunological responses. Swine lungs were collected from 4-6-week-old germ-free pigs by transport medium from Animal husbandry House Coimbatore, Tamil Nadu, India to Animal Tissue Culture Laboratory, Kongunadu Arts and Science College, Coimbatore, India.

Establishment of Swine Respiratory Epithelial Cells: Lungs were washed with 1× PBS buffer and cut into small pieces of 1 mm 3a nd incubated with collagenase for 2 hrs 37 °C then, centrifuged at 500 rpm 5 min.

The cell pellet was washed two times with 1X PBS and a seed on T-flask contains Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 µg/ml penicillin and Streptomycin. Cell culture was performed under standard conditions (37 °C, 5% CO_2, 90% humidity). After 24 h, non-adherent cells were transferred to another flask and cultured same condition [20, 16].

Cytoprotective Activity: MTT assay is a test to check metabolic activity of proliferating cells under *in-vitro* conditions [20]. Aliquots of 5000-10000 cells were loaded on each well of the microtitre plate. After seeding the cells, incubate the plate for 24 h. After incubation, various concentrations of extract (100-500 mg/ml) were added, DMSO was taken as negative control, and Levofloxacin (Drug) as positive control.

Keep the plate for 24-8 h of incubation, then 10 µl MTT reagent (5 mg/mL) was added in each well and plates were kept for 1 h in incubator. After 1 h of incubation, the MTT reagent was removed and 100µl of isopropanol was added to each well.

The intensity of the purple formazan solution was measured at 594 nm in a spectrophotometer. Each experiment was performed in triplicates, and the same protocol was followed until the completion of the experiment.

Biocompatibility Assay: Hemolysis experiment was carried out as described by [21]. Different concentrations (100-500 mg/mL) of *T. chebula* were incubated separately with 50 µl of 5% (v/v) red blood cells (RBC) in50 mM PBS (pH 7.2) at 37 °C for 1 h with shaking (150 rpm).

TritonX-100 and 50 mM PBS (pH 7.2) served as positive and negative controls, respectively. At the end of incubation, the hemolytic activity was measured spectrophotometrically at 540 nm using a plate reader, and the percentage of hemolysis was determined using the following equation:

$$\% \text{ of hemolysis} = EX - NC \times 100$$

PC.NC Where, EX = Absorbance of test samples (Extract concentration at 100 - 500 mg/ml + RBC) NC =Absorbance of negative control (PBS + RBC) PC = Absorbance of positive control (Triton X-100 + RBC)

RESULTS AND DISCUSSION:

Phytochemical Analysis: The presence of various phytochemicals was analyzed qualitatively by various standard methods based on chromogenic reactions. The methanolic extract of *T. chebula* showed the presence of important secondary metabolites such as tannins, alkaloids, terpenoids, phenol, and flavonoid. However, glycoside, steroid, saponin, Anthraquinone, and Cardio glycoside were absent. The methanolic extract gave a highly intense chromogenic reaction, suggested that methanol solvent extraction contains more phytochemicals **Fig. 2**. According to 20, methanolic extract of *T. chebula* contains alkaloids, terpenes, steroids, flavonoids, tannins, and phytophagous. Similar to our study, an abundant quantity of flavonoids and alkaloids were present in the extract reported by [22].

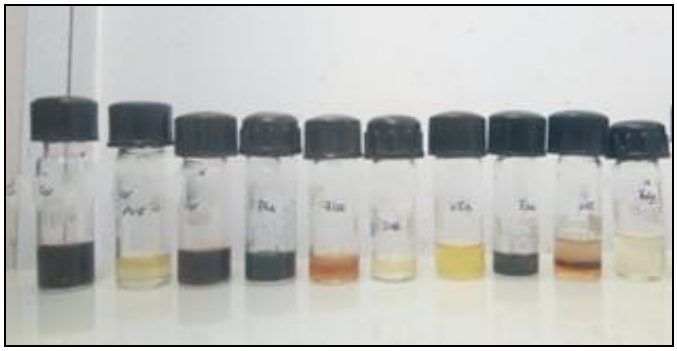


FIG. 2: PHYTOCHEMICAL ANALYSIS OF *T. CHEBULA*

Characterization Study: To analyze the contents of the extract, FTIR was used and the resulting spectra were shown in **Fig. 3** and **Table 1**. The peak of 3346.56 cm^{-1} in the spectra corresponds to O–H stretching indicating the presence of alcohols. The peak at 1605.38 cm^{-1} was assigned as N–H bend indicates the presence of primary amines. The peak at 1029.27 cm^{-1} in the spectra corresponds to the C–N stretching of aliphatic amines were observed. The peak at the 747.65 cm^{-1} region could be attributed to C–H, which is the characteristic of polyphenols.

It is interesting to note that the presence of various compounds exhibits effective antimicrobial action, besides the ability to form complexes with soluble proteins and with bacterial cell walls as well. It possesses very good antioxidant activity. It has been reported previously by 23 that methanolic extract if *T. chebula* exists with two peaks at 2921.70 and 2850.80 cm^{-1}.

The spectra of herbal ethanolic extract indicated the presence of various functional groups such as alkanes, alkynes, carboxyl esters, and polyphenols indicated to be effective antimicrobial and antioxidant stress [24]. The spectral analysis of *T. superba* was done by [25].

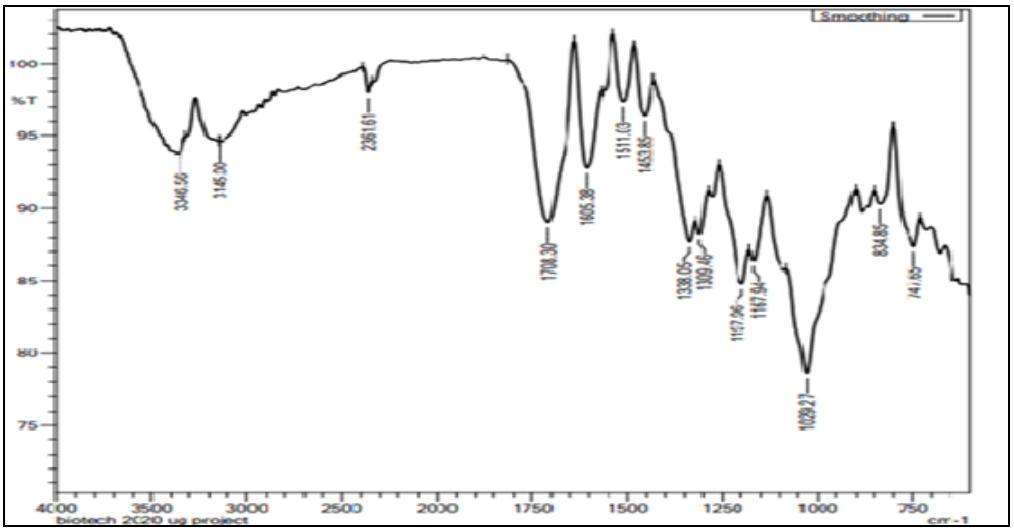
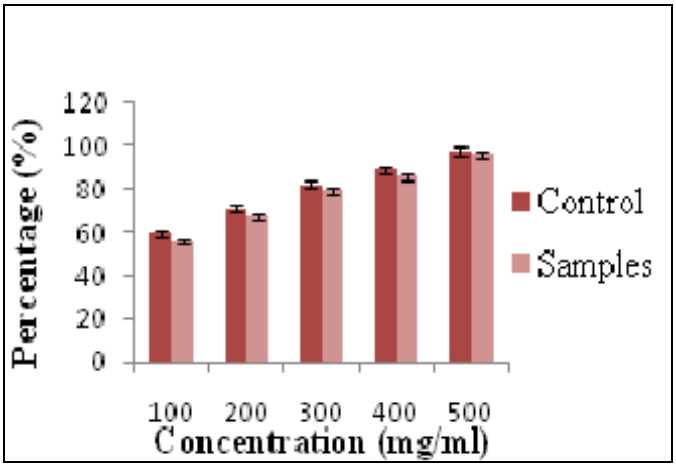


FIG. 3: FTIR ANALYSIS OF *T. CHEBULA*

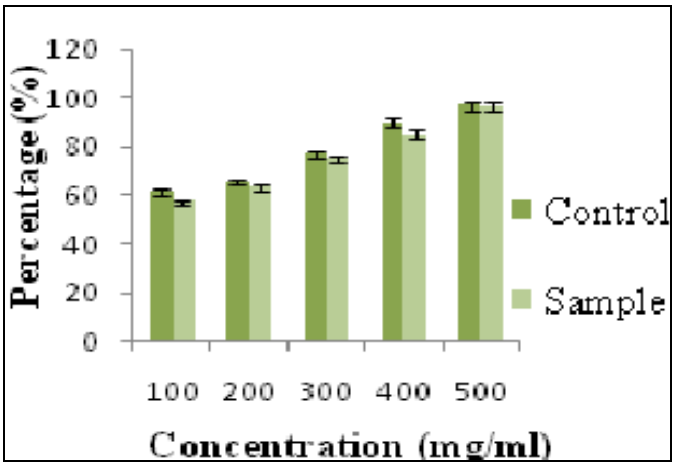
TABLE 1: DETERMINATION OF FUNCTIONAL GROUPS IN FTIR ANALYSIS

Frequency (cm^{-1})	Type of vibration &bond	Functional group(s)	Uses
747.65	C–H	Polyphenols	Reduce oxidative stress
1029.27	C–N stretch	Aliphatic amines	Catalyst
1605.38	N–H bend	Primary amines	Relieve allergic disorder
3346.56	O–H stretch	Alcohols	Bactericidal activity

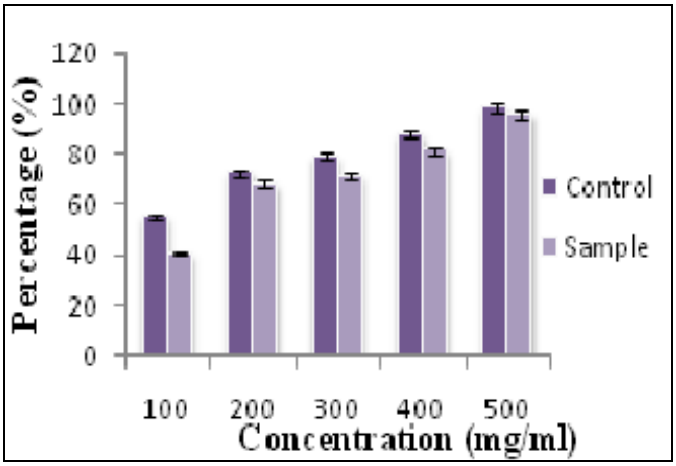
Antioxidant Study: Antioxidants act as a major defense against radical-mediated toxicity by protecting the damages caused by free radicals. The antioxidant activity of the methanolic extracts of *T. chebula* was also evaluated by FRAP, DPPH and hydroxyl radical scavenging activity. The results were compared and described in Figures 4a, b and c. The reduction percentage was plotted against the concentration of the sample. All three assays showed maximal scavenging activity of 96.6, 96.2, and 95.8%, respectively, at 500 mg/ml concentration. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radicals. The lower the IC_{50} value, the higher the anti-oxidant activity of samples. The results revealed that the reducing activity significantly increased as the concentration of the extract was increased at 500 mg/ml. From observations, it is inferred that *T. chebula* showed similar and satisfactory results in comparison with standard ascorbic acid. Values are expressed as mean ± SD.



A. FRAP



B. DPPH



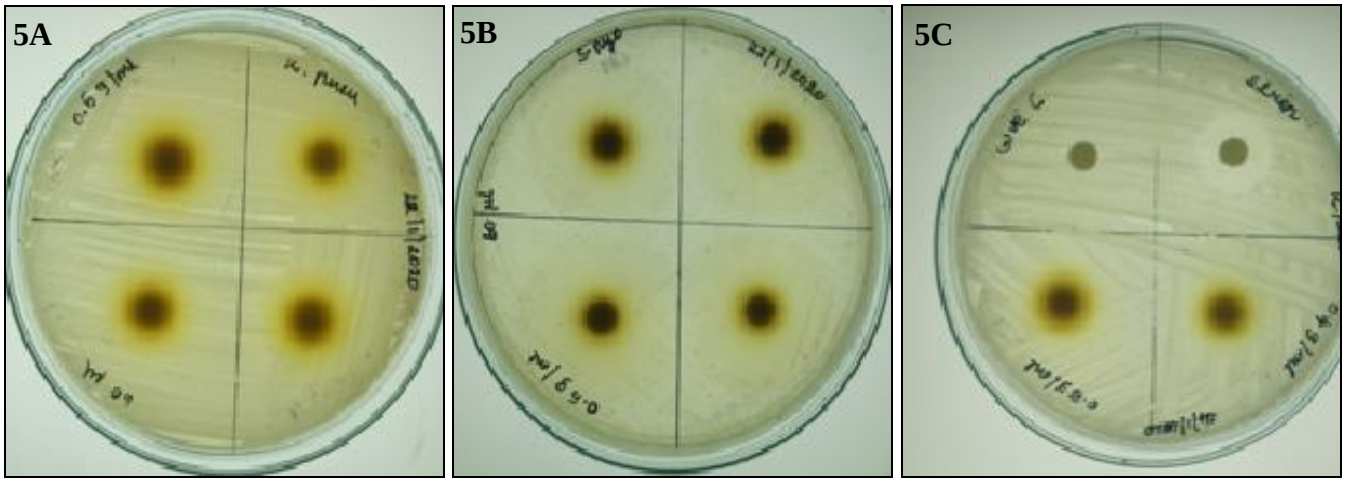
C. HYDROXYL RADICAL SCAVENGING ACTIVITY

FIG. 4: ANTIOXIDANT ACTIVITY OF *T. CHEBULA*

The reductive capacity of a compound depends on the presence of reductones, which exhibits anti-oxidative potential by breaking the free radical chain and donating a hydrogen atom. Therefore, reducing activity leads to the termination of the radical chain reactions. The presence of antioxidant reductants in the methanolic extract of *T. chebula* causes the reduction of the Fe3+/ ferricyanide complex to the ferrous form, indicating that the extract of *T. chebula* has significant reducing power similar to the standard. It has been reported the existence of a similar linear co-relationship between the reducing power and methanolic content [26]. According to the method of 27, the phenolic extract of *T. chebula* exhibit the highest scavenging activity at 150 µg/ml with I_{C50} value of 14 ± 0.05 µg/ml. DPPH is the stable, nitrogen-centered free radical, which accepts hydrogen from the antioxidants present in the extract converted into a molecule diphenyl-picryl hydrazine [28, 29].

The observed reduction of DPPH by the extract either due to transfer of hydrogen atom or electron. These methanolic extracts are effective hydrogen donor, which makes them very good antioxidants [21]. *T. chebula* showed significant scavenging activity of H_2O_2 in a concentration-dependent manner. Hydrogen peroxide is a weak oxidizing agent and directly inactivates a few enzymes, usually by oxidation of essential thiol groups. It can cross cell membranes rapidly, and once inside the cell, H_2O_2 likely reacts with Fe^{2+} and possibly Cu^{2+} ions, to form hydroxyl radicals, which then become powerful oxidizing agents. This methanolic extract of *T. chebula* acts free radical scavengers because of their hydrogen donating and scavenging ability [25].

Antibacterial Activity: The antibacterial activity of methanolic extract of *T. Chebula* was tested at various concentrations and the zone of inhibition was presented in **Table 2**. The largest zone was recorded against *K. pneumoniae*, and it was found as 20 ± 0.43 mm at the concentration of 500 mg/ml **Fig. 5A**, followed by *S. pyrogens* (15 ± 0.38 mm; **Fig. 5B** and *S. pneumonia* (14 ± 0.29 mm; **Fig. 5C** respectively at the same concentration. Whereas positive control and the standard showed 19 ± 0.82 and 17 ± 0.17 mm respectively against *K. pneumoniae*. Hence, it can be expected that the plant extract possesses various phytochemicals which might be responsible for the inhibition of bacterial metabolism.



K. PNEUMONIAE *S. PYOGENES* *S. PNEUMONIAE*

FIG. 5: ANTIBACTERIAL ACTIVITY OF THE PLANT EXTRACT AGAINST

Bacterial Pathogens:

TABLE 2: ZONE OF INHIBITION BY AGAR WELL DIFFUSION METHOD

Test organisms	Standard (500mg)	Positive Control	Concentration (mg/ml)				
			100	200	300	400	500
K. pneumoniae	17±0.17	19±0.82	9±0.04	11±0.41	14±0.20	18±0.35	20±0.43
S. pneumoniae	18±0.82	19±0.96	7±0.47	8± 0.36	11±0.36	13±0.24	14±0.29
S. pyogenes	18±0.17	18±0.12	8±0.32	8±.0.20	12±0.21	13±0.37	15±0.38

Values are represented as mean ± S.D

Similar to our finding, highest antibacterial activity was observed against Enterobacter aerogenes treated with fruit extracts of *T. chebula* [30].

Besides, the research work of 31, depicted that greater zones of inhibition was observed din all the 3 forms (Powder, Water and Concentration) against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

MIC and MBC: Summing up the results of MIC and MBC, *T. chebula* extract was active against *K. pneumonia* at a varying concentration (100 – 500 mg/ml) tested with standard levo floxacin **Fig. 6**. It is evident that *T. chebula* at a minimum concentration (100 mg/ml) inhibited bacteria growth at 38%, which was very close with the standard treated at the same concentration, with the percentage of 40%. As mentioned in the materials and methods, MBC was performed from the MIC

96-well plates. The concentration of the extract *T. chebula* used in MBC assay ranged between 100-500 mg/ml.

The results revealed that no bacterial growth was observed on MHA plates in both extract **Fig. 7A** and standard **Fig. 7B** at 100 mg/ml during 48 h of incubation. From the findings of 32, Sequential extraction of all aqueous and methanol extracts of *T. bellirica* displayed antibacterial activity (MIC 0.25–4 mg/mL) against all strains of methicillin-resistant *Staphylococcus aureus* (MRSA), Acinetobacter spp. and *P. aeruginosa*.

MBC of alkaloids of *T. chebula* range was recorded highest at 256.41 ml/g against *E. aerogens* and MIC against *A. tumefaciens* was found at 625 mg/ml [30].

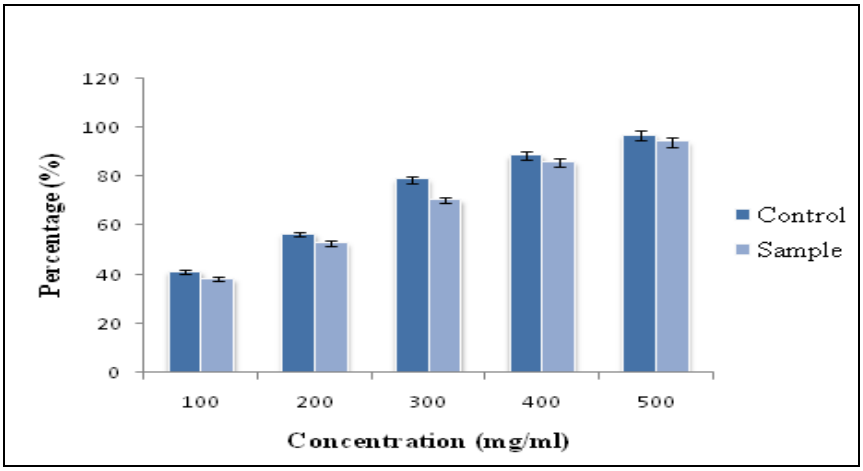


FIG. 6: MIC OF EXTRACT AND STANDARD

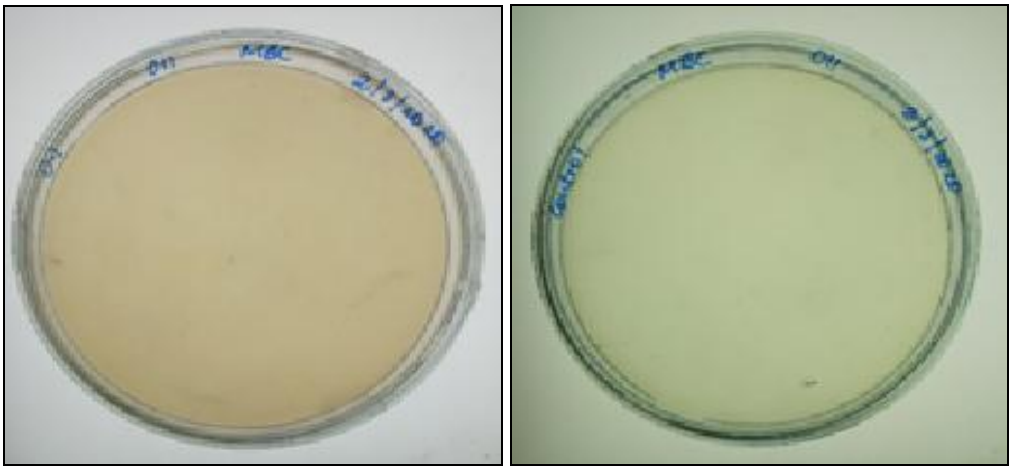


FIG. 7: MBC OF EXTRACT AND STANDARD

Time Kill Assay: The time kill profile of methanol extract of *T. chebula* (100 mg/ml) against the test organism *K. pneumonia* showed gradual rise up of reduction in number of viable cells till 20 h of incubation and the reduction was found as 75% after that, the reduction in number of viable cells found stable till 24 h.

OD was taken at a regular 4 h of time interval. When compared to standard, reduction in viable cells is higher at 20 h with the reduction percentage of 80, but the reduction in number of viable cells found rise up till 24 h. Time-kill assay studies showed that the extracts possess bacteriostatic action **Fig. 8**. Similarly, time-kill kinetics studies indicate that methanol extracts of *T. gibbosa*, *T. elegans*, *S. commune*, and *V. volvacea* exhibited bacteriostatic actions against gram-positive and negative organisms [19]. In the study of 33, phenolics of chebulic myrobalan (CM), determined to have strong antibacterial activity, were tested for the rate of killing bacteria in a given time (kill kinetics) against methicillin-resistant *Staphylococcus aureus* (MRSA) and trimethoprim-sulphamethoxazole (SXT/TMP)-resistant uropathogenic *E. coli*.

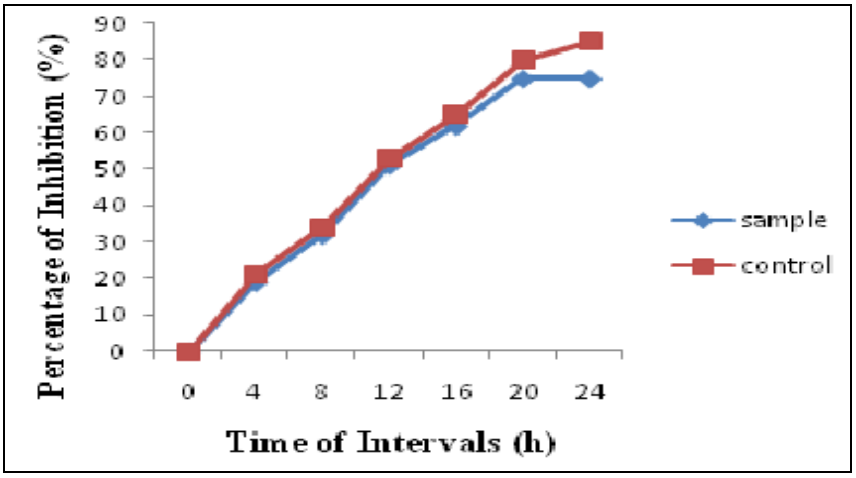


FIG. 8: TIME KILL PROFILE OF METHANOL EXTRACT *OF T. CHEBULA*

Isolation and Establishment of Lung Cell: The isolation procedure of the swine epithelial cells derived from the lungs is described in detail in the Materials & Methods section. The cell clusters were observed under an inverted microscope and shown in **Fig. 9**. The cells were attached uniformly on T-25 flasks reached 80-90% confluence by 48 h. The fibroblasts were removed by treating the cell monolayer with 0.03% trypsin for 3 min, every 48 h followed by PBS wash and the addition of fresh media. Primary respiratory epithelial cells were monitored daily until the cells reached confluence. These cells could be sub-cultured in normal or collagen-coated tissue culture flasks. The sub-cultured cells attached to the tissue culture surface in 24-48 h. At later passages, some cells appeared irregularly sized, indicative of cell differentiation.

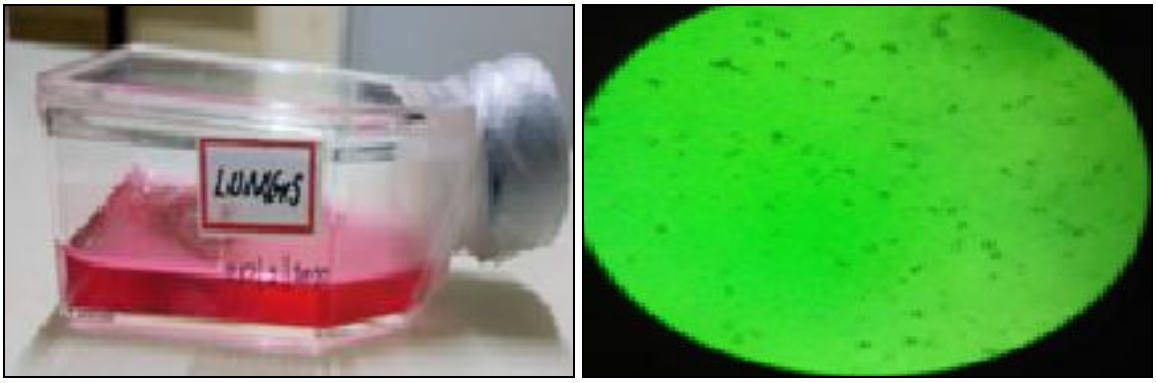


FIG. 9: CLUSTERED LUNG EPITHELIAL CELLS UNDER INVERTED MICROSCOPE

MTT Assay: Swine Lung Cell lines-based cytotoxic assay with different concentrations of the methanolic fruit extract was performed with standard drug levofloxacin. Initially, the viability of the cell was more at 100 mg/ml concentration of the extract, the cells start to decrease gradually from 200 -500 mg/ml. It shows increasing the concentration of the extract decreases the viability of the cells. Whereas, with the standard the viability of the cells were stable up to 300 mg/ml concentration presented in **Fig. 10**. The percentage viability was found to be 98.5 ± 0.12, 96.8 ±0.10 and 95.5 ± 0.12 respectively at 100, 200 and 300 mg/ml plant concentration. The percentage viability was reached a maximum at 99.3 ± 0.15 up to 300 mg/ml concentration of standard drug levofloxacin. The result was found to be statistically significant. In this finding, the standard drug levofloxacin showed comparatively better results than *T. chebula*. But it causes serious side effects also, skip doses leads bacteria may become resistant to antibiotics. The herbal extract has a potential effect against *K. pneumonia* and could be an exquisite alternative to these synthetic agents. Recently, researchers focused on the holistic system for treating disease than allopathy due to many side effects and antibiotic resistance. The research finding of 34 proposed that herbal teas combination with Rosehip, cinnamon, black and green tea reduce the severity of disease-causing clinical

isolates *P. aeroginosa, S. aureus* and *K. pneumonia* than the standard anti-biotics ciprofloxacin, erythromycin and amikacin tested. In another study, the anti-proliferative effect was carried out for the methanolic fruit extract of *T. chebula* against oral cancer cell lines at different concentrations to determine the growth inhibition by MTT assay [35].

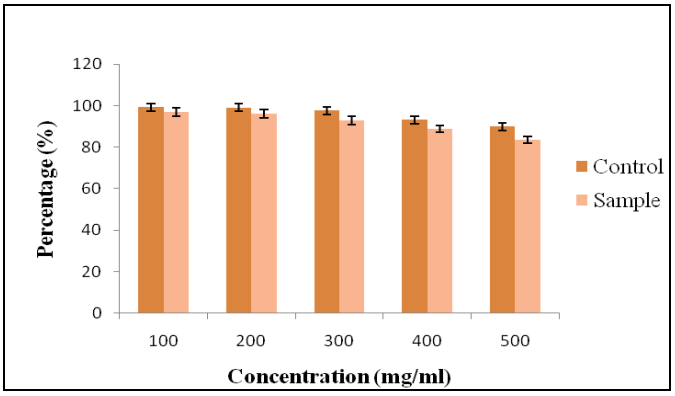


FIG. 10: DETERMINATION OF CELL VIABILITY

Biocompatibility Assay: Each percentage represents the mean values of samples. Different concentration of *T. chebula* extract and standard levo floxacin (100- 500 mg/ml) was used to perform this assay to determine the bio compatability. Hemolysis was induced by Triton X-100.At 100 mg/ml concentration, 99.2 and 97.3 % of viable cells were recorded when treated with *T. chebula* extract and standard levo floxacin respectively. At an increasing concentration of *T. chebula* extract, number of viable cells may decreased, in contrast, stable viability recorded with standard at increasing concentration. So the minimal hemolytic effect and bio compatibility on human red blood cells was found as 100 mg/ml for *T. chebula* extract **Fig. 11**.

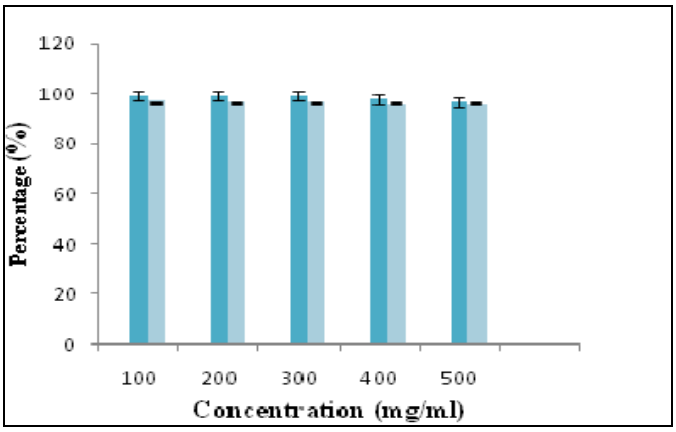


FIG. 11: BIOCOMPATIBILITY ANALYSIS OF *T. CHEBULA*

A bio-compatible biomaterial does not have toxic or injurious effects on biological systems. The study carried out by 36, explained the importance of biocompatibility in the modified biomedical-grade chitosan derivatives examination *in-vitro* in order to produce high-quality, biocompatible dressings. The compatibility was performed with the extracts of *T. chebula* on Proliferation of Keratinocytes and Fibroblasts Cells for wound healing which showed the mimimal hemolytic activity in the tested RBC [37].

CONCLUSION: The present study disclosed that the methanolic extract of *T. chebula* exhibited the presence of various secondary metabolites. The performed study proved to have strong antibacterial and antioxidant properties. Hence, it may be used as a potential source of natural antibacterial and antioxidant agents. Also, the existing antibiotics against respiratory infections are gradually becoming ineffective against multidrug-resistant pathogenic bacteria, so the new and alternative sources for future antibiotics may be explored well in advance. Results of the study reveal that *T. chebulacan* be a potential candidate against respiratory infection causing pathogenic bacteria.

ACKNOWLEDGMENT: The authors like to thank Kongunadu Arts and Science College and DST-FIST for providing the laboratory facilities; also, we acknowledge the Animal husbandry House Coimbatore, Tamil Nadu, India.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest for this study.

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How to cite this article:
Anasuya S, Vishnu PB and Gunasekar A: Pharmacognostical evaluation of *Terminalia chebula* fruit extract against respiratory tract infectious pathogens. Int J Pharm Sci & Res 2021; 12(10): 5446-57. doi: 10.13040/IJPSR.0975-8232.12(10).5446-57.