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Original Research Article

Antimicrobial Effect of *Cinnamomum verum* Leaf Extract on Fungus and MDR Bacteria

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Abstract: Multi-drug resistance is a major concern to medical science across the globe. The inability of most drugs to destroy different pathogenic bacteria and fungi demands the use of natural products like phytochemicals to treat various infections. At this juncture, *Cinnamomum verum* is an active agent against various bacteria and fungi. *C. verum* or true cinnamon is widely used as a spice. It has several medicinal values and is widely accepted as traditional medicine. Cinnamaldehyde is the most active compound of *C. verum* that is responsible for its antimicrobial activity. In this study, the minimum inhibitory concentration (MIC) of C. *verum* leaf ethanolic extract was determined against different bacteria and fungi along with ethanol as a control. The extract showed remarkable antifungal action on *Candida albicans* and antibacterial action against *Pseudomonas aeruginosa* with a MIC value of only 0.039 mg/ml. The MIC value against other microorganisms ranges from 0.78 mg/ml to 25mg/ml. *C. verum* leaf extract can be used as an effective antimicrobial agent in fungal and bacterial infections.

Keywords: *Cinnamomum verum*, Cinnamaldehyde, Antimicrobial activity.

INTRODUCTION

An increase in multidrug Resistant bacteria and fungi poses a major threat to medical science and human health. Patients acquiring infections caused by MDR strains are difficult to treat and in many cases lead to death. These infections are mostly hospital-acquired and are of major concern. To treat these pathogens an alternative to conventional drugs is being looked upon. Various phytochemicals have been found to have antimicrobial effects [1]. These compounds sometimes show high efficacy against MDR strains alone or in synergy with antibiotics. Among various plants and phytochemicals, *Cinnamomum verum* is one such plant whose extract is effective against various bacteria and fungi.

Cinnamomum verum is a tropical plant (Fig 1) native to Sri Lanka and found in southern parts of India. It is widely used as a spice and as a traditional medicine [18]. It belongs to the family Lauraceae [19]. Cinnamaldehyde (Fig 2) is one of the most active compounds of *Cinnamomum verum*. It has a potential antimicrobial activity. It is effective against a wide variety of bacteria and fungi. A naturally occurring flavonoid gives the spice its flavour and colour. It contains two unsaturated functional groups of aldehyde and carbon-carbon double bonds [21].

Cinnamomum verum has been proven a potent source of antimicrobial agent. The extract of different components of this plant has antiviral, antibacterial and antifungal activity. It is also reported to be a good pesticide. Thus under the current scenario where multi-drug resistance is a major concern; this can be used as an alternative therapy. Besides the antimicrobial activity of cinnamaldehyde, the active component of this plant has effective antidiabetic, antipyretic, anti-inflammatory, antioxidant, antitumor, cholesterol lowering, cardio protective, anti-parkinsonism activities [7-12]. It can be effectively used for the treatment of osteoporosis.

Various studies have shown the efficacy of *Cinnamomum verum* bark and leaf extracts on different organisms. *C. verum* fresh leaf extract was shown to be effective against *Enterococcus faecalis* [16]. Essential oils obtained from fresh leaves of *C. verum* were shown to be effective against *Streptococcus mutans* and *Lactobacillus acidophilus* causative agents of dental plaque. The MIC values obtained for *Streptococcus mutans* were less than that of gentamycin, thus proving the higher efficacy of Cinnamon leaf extract essential oil than gentamycin [2]. Cinnamaldehyde is reported to have high antibiofilm activity on MRSA [17] and *E coli* [4]. The antifungal effect of *Cinnamomum* extract on azole-resistant *Candida* sp. is effective in reducing *Candida* biofilm. In this study, we explored the leaf extract of this

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plant against fungus and bacteria including some MDR microorganisms.

Fig 1: The *Cinnamomum verum* plant

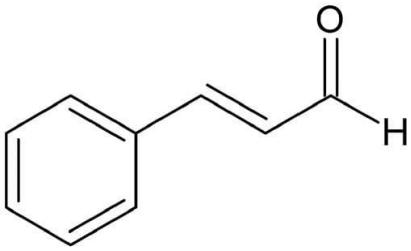

Fig 2: Chemical structure of cinnamaldehyde (20)

MATERIAL AND METHODS

Dry *Cinnamomum verum* leaves were taken. The leaves were crushed into powder. 1gm of the powder was weighed using an analytical balance and suspended in 10ml of ethanol (100mg/ml) for 48 hours (Fig. 3). After 48 hours, the suspension was centrifuged and the supernatant was separated. UV-spectra of the supernatant showed a characteristic peak of cinnamaldehyde (Fig.4) MIC value was checked in a 96-well plate. Bacterial isolates were made into 0.5 McFarland opacity bacterial suspensions using normal saline (NS) with DensiCHEK. Normal saline was used to maintain the tonicity of the medium and to prevent lysis of bacterial cells. At first 100 µl of Muellar-Hinton broth was added to each well of the plate. 100 µl leaf extract was added in the first well and then serially diluted along the horizontal row up to 8 wells and then 100 µl of the excess fluid was discarded from the eighth well. Thus in each step, there was a double dilution of the extract until the eighth well. In another row, similar dilutions were made with vehicle alcohol as control and the bacterial suspensions were similarly added. In this way, in different rows, different bacterial and fungal suspensions were added. Two blanks were taken, one of extract and one of ethanol. 10µl of the bacterial or fungal culture of 0.5 McFarland concentration was added in each well leaving the blanks. Two fungal strains were taken, *Candida albicans* and *Candida parapsilosis*. Six different bacterial strains were taken, *Escherichia coli* ATCC 25922, *Escherichia coli* (MDR), *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* (MDR), *Pseudomonas aeruginosa* (MDR). Absorbance was measured at 620 nm as a 0-hour reading and kept for overnight incubation at 37^{o} C. After 24 hours of incubation, absorbance was measured again at 620nm. The absorbance obtained at 0 hours was subtracted from that obtained after 24 hours. The absorbance of the blanks was subtracted from the respective wells. Graphs were plotted to have the X-axis as concentration and the Y-axis as absorbance. MIC values were determined from the graphs and noted.

Fig 3: Crushed leaves of *Cinnamomum verum* and the ethanolic extract of the leaves

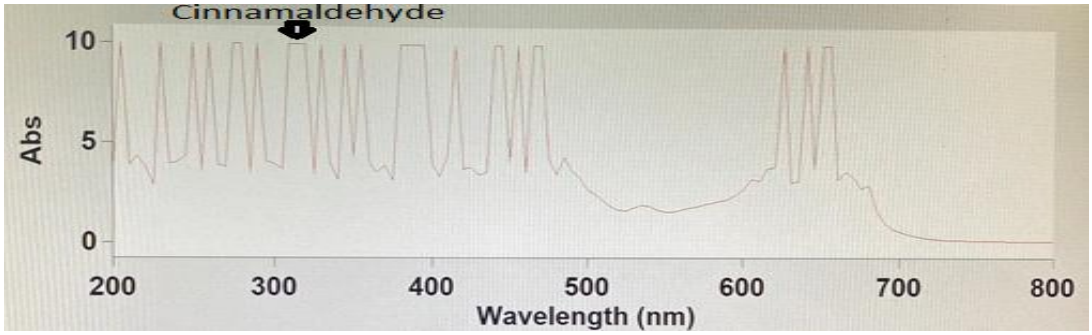

Fig 4: Absorption spectra of *Cinnamomum verum* leaf extract showing the absorption maxima of cinnamaldehyde

RESULTS

The results of this experiment are Given in the Fig. 5-12. MIC value of the extract against *C. albicans* and *Pseudomonas aeruginosa* was only 0.39 mg/ml. MIC value in case of *C. parapsilosis* was 6.125 mg/ml, MIC value against *E. coli* MDR was 0.78 mg/ml. MIC value against other organisms studied by us was 25 mg/ml.

Effect of *Cinnamomum verum* leaf extract on *Candida albicans*

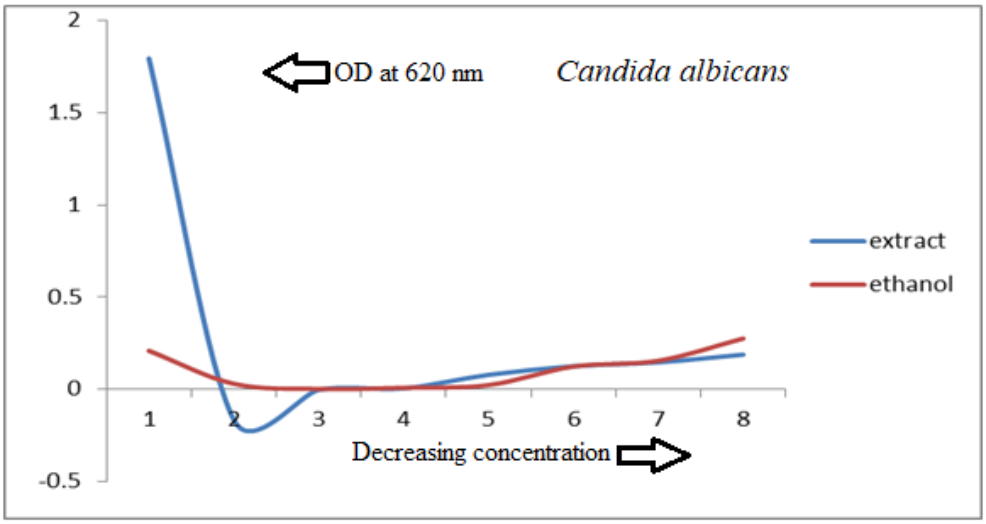

Fig 5: Graph showing effect of *Cinnamomum verum* leaf extract on growth of *Candida albicans* (MIC value: 0.039 mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of Cinnamomum verum leaf extract on *Candida parapsilosis*

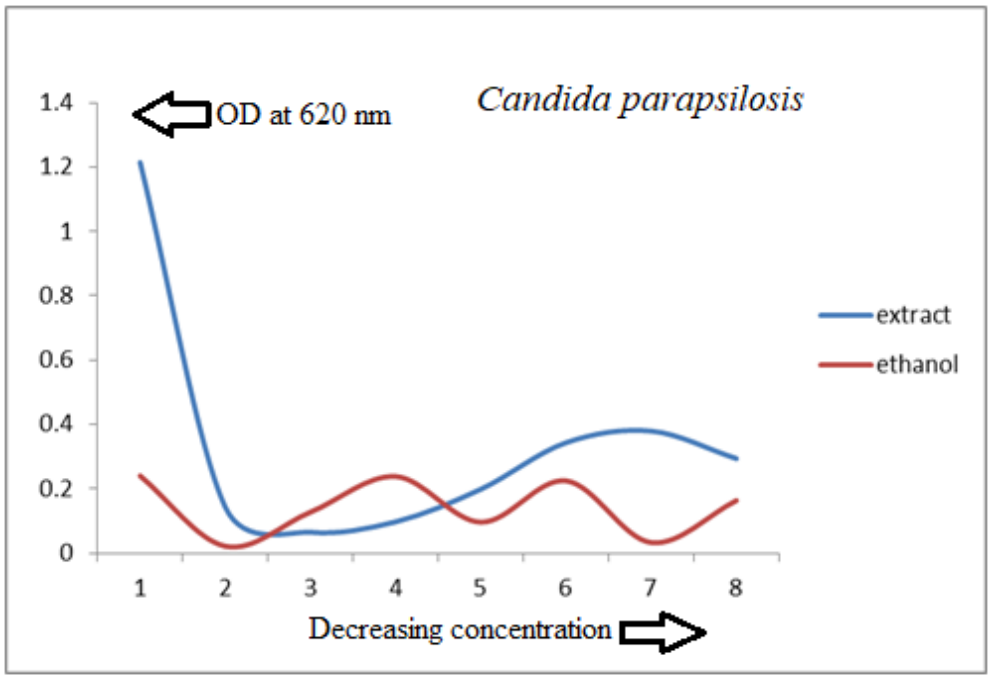

Fig 6: Graph Showing effect of *Cinnamomum verum* leaf extract on growth of *Candida parapsilosis* (MIC value is 6.125mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of *Cinnamomum verum* leaf extract on *Escherichia coli* ATCC 25922

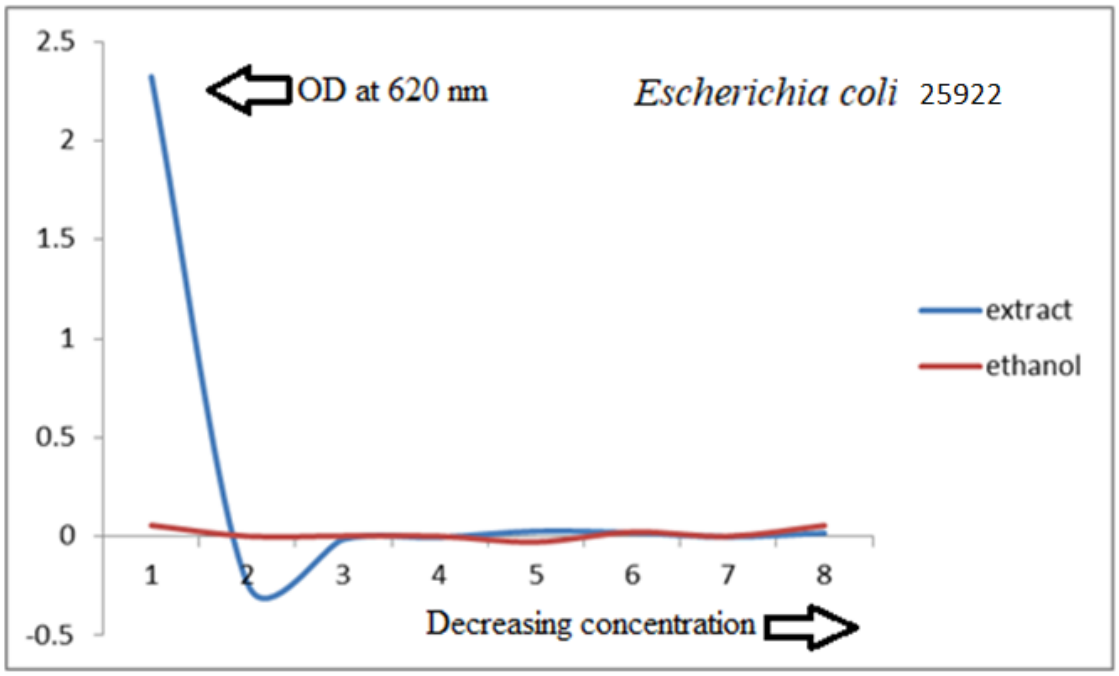

Fig 7: Showing effect of *Cinnamomum verum* leaf extract on growth of *Escherichia coli* ATCC 25922 (MIC value is 25mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of *Cinnamomum verum* leaf extract on *Escherichia coli* MDR

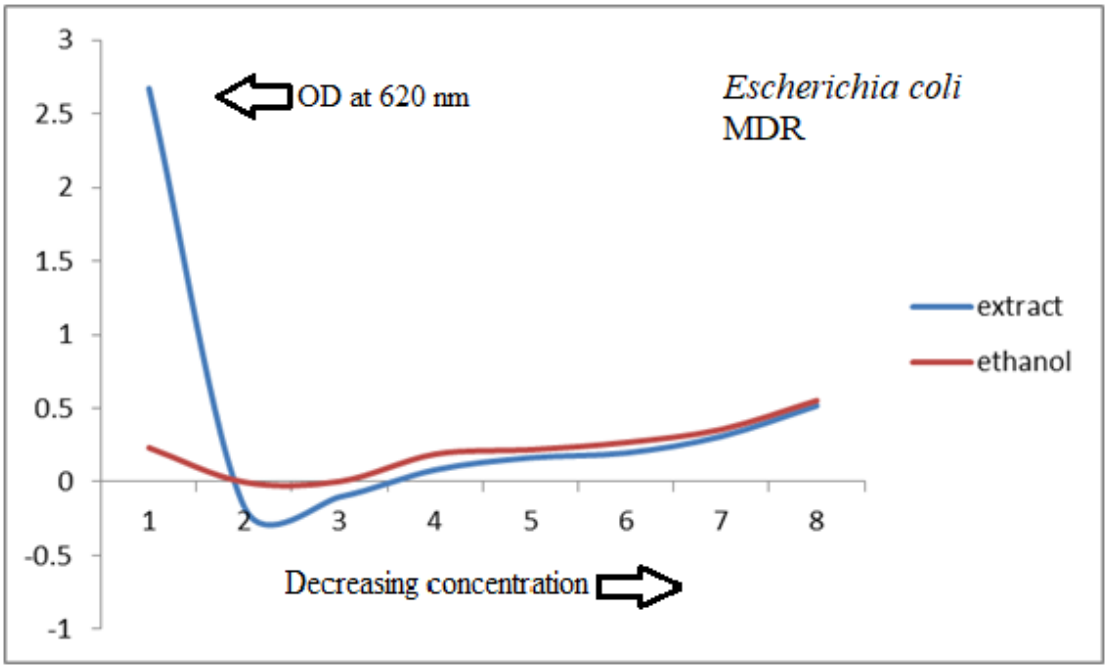

Fig 8: Showing effect of Cinnamomum verum leaf extract on growth of *Escherichia coli* MDR (MIC value is 0.78125 mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of Cinnamomum verum leaf extract on *Staphylococcus aureus* ATCC 25923

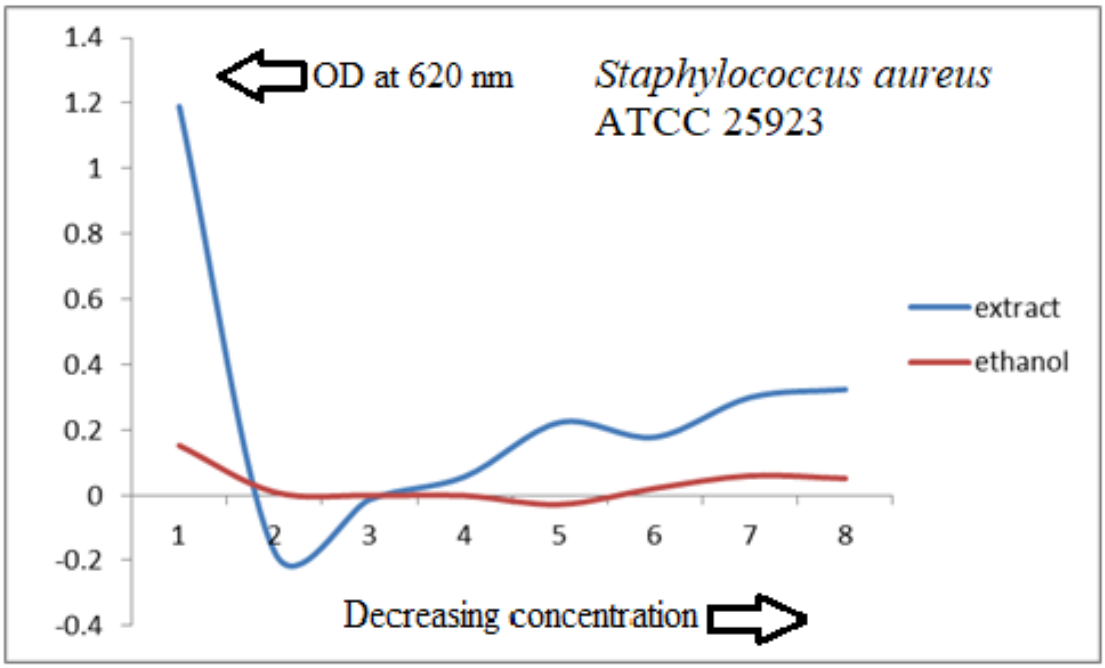

Fig 9: Showing effect of *Cinnamomum verum* leaf extract on growth of *Staphylococcus aureus* ATCC 25923 (MIC value is 25mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of *Cinnamomum verum* leaf extract on *Staphylococcus aureus* MRSA

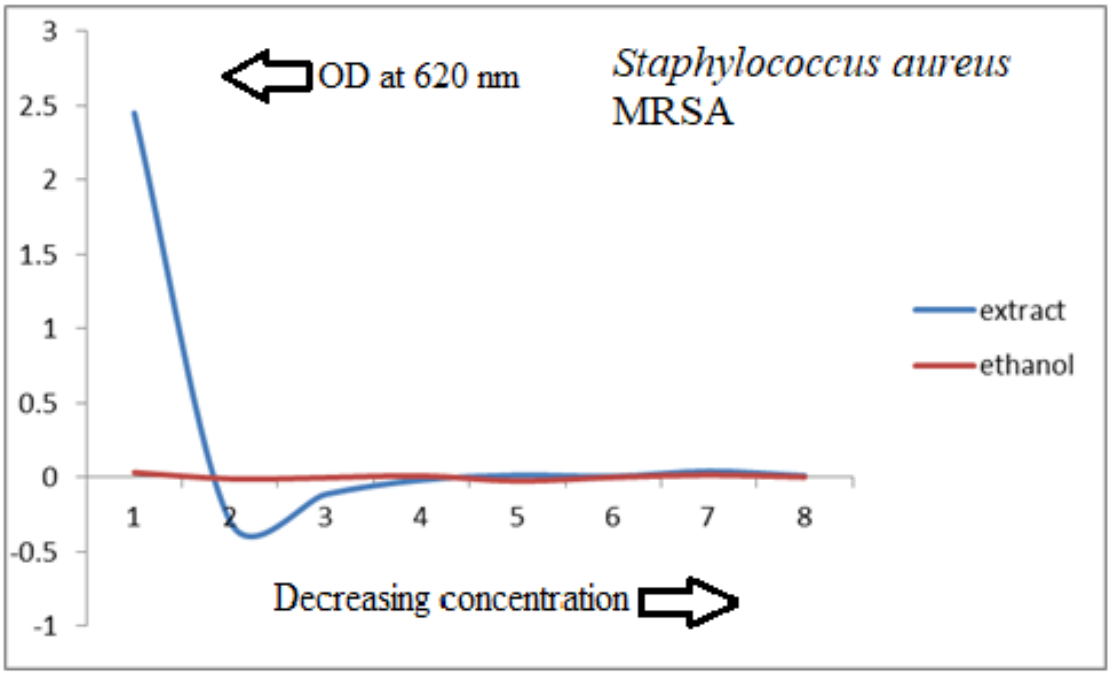

Fig 10: Showing effect of *Cinnamomum verum* leaf extract on growth of *Staphylococcus aureus* MRSA (MIC value is 25mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of Cinnamomum verum leaf extract on *Klebsiella pneumoniae* MDR

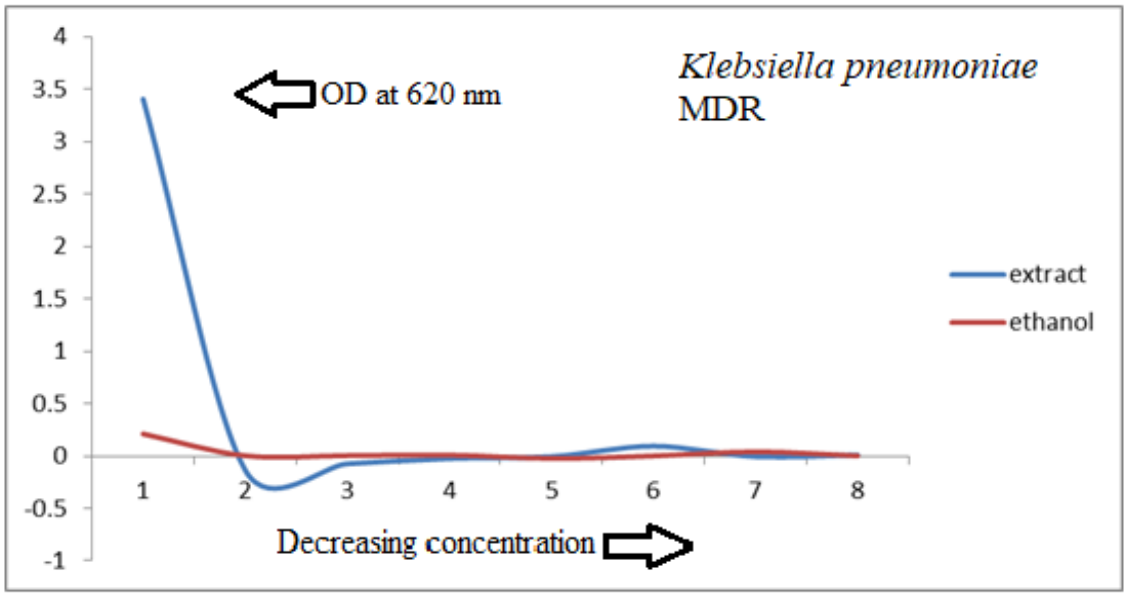

Fig 11: Showing effect of *Cinnamomum verum* leaf extract on growth of *Klebsiella pneumoniae* MDR (MIC value is 25mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

Effect of *Cinnamomum verum* leaf extract on *Pseudomonas aeruginosa* MDR

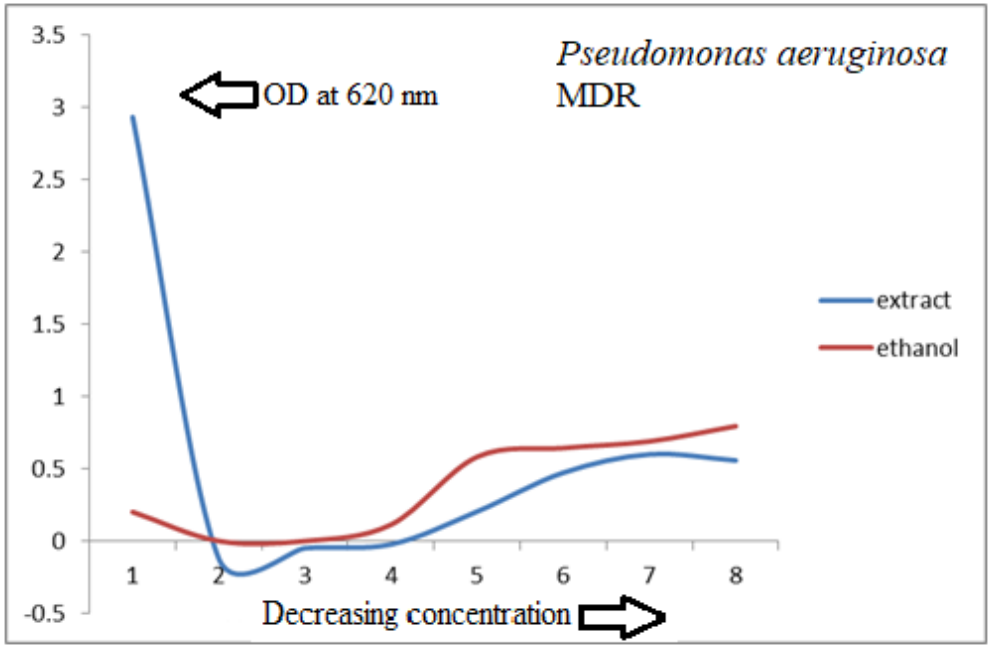

Fig 12: Showing effect of *Cinnamomum verum* leaf extract on growth of *Klebsiella pneumoniae* MDR (MIC value is 0.0396875mg/ml). 1-50mg/ml, 2-25mg/ml, 3-12.5mg/ml, 4-6.125mg/ml, 5-3.125mg/ml,6-1.5625mg/ml, 7-0.78125mg/ml, 8-0.39625 mg/ml

DISCUSSION

With the increasing resistance of bacteria and fungi to various drugs, different phytochemicals come into remedy. In this experiment, we have studied the antibacterial and antifungal effects of *Cinnamomum verum* leaf extract. The results shown in Fig. 5-12 show the MIC values of the extract. The two fungal species studied in this experiment ie., *Candida albicans* and *Candida parapsilosis* show different degrees of sensitivity as the MIC values vary from 0.0396875mg/ml in *Candida albicans* to 6.125mg/ml in *Candida parapsilosis*. The different bacterial species also show different MIC values in the extract. When compared between *Escherichia coli* ATCC 25922 and *Escherichia coli* MDR *E. coli* ATCC seems to be more resistant to extract than *E coli* MDR as the MIC values obtained were 25mg/ml and 0.78125mg/ml respectively. In the case of *Staphylococcus aureus*, both the ATCC strain and the MRSA strain show an MIC value of 25mg/ml. this indicates that *Staphylococcus aureus* is relatively resistant to the extract. Similar resistance was also found against multi-drug resistant (MDR) strains of *Klebsiella pneumoniae*. Paradoxically MDR strain of *Pseudomonas aeruginosa* shows high sensitivity to the extract with an MIC value of 0.39625 mg/ml.

The main component of *C verum* leaves is trans-cinnamaldehyde. This compound has several antimicrobial and antifungal effects. The aldehydic group of cinnamaldehyde is absorbed by the hydrophilic groups of the bacterial cell surface and allows it to pass through it and disrupt the permeabilization of the cell wall, thus spilling out the inner components. Cinnamaldehyde has been seen to damage the cell wall and alter its permeability along with oxidative damage [3,6] in *Staphylococcus aureus*. It is also seen that cinnamaldehyde downregulates transcription and translation of the mecA gene of MRSA [4]. Cinnamaldehyde is also reported to alter cell surface morphology, induce cell shrinkage and lowering cytoplasm in *E coli* and *Klebsiella pneumoniae* [5].

CONCLUSION

Cinnamomum verum leaf ethanolic extract shows considerable antimicrobial activity against some microorganisms including MDR strains. Thus it can be used as an alternative to drugs for the treatment of MDR bacteria and fungi. Although further studies need to be conducted, as of now it can be said that *Cinnamomum verum* leaf extract is an effective antimicrobial agent.

Conflict of Interest: The author declares no conflict of interest.

Author's Contribution

Tanni Datta under supervision of Arup Kumar Dawn carried out the experiment. Satadal Das designed the study procedure and analysed the data. Bhaskar Narayan Chaudhuri and Partha Guchhait reviewed and edited the manuscript.

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