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Research Article

Inhibitory Effect Of Natural Extracts Against Bacteriophages Towards Evaluation Of Their Anti-Viral Potential

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ABSTRACT

Viral infections have caused wide-scale suffering and death globally throughout the history, very recent being the deadly Covid-19 infection. Many bacterial infections also result in various serious diseases. It is well known that microbial, especially viral infections are mainly transmitted between individuals through the respiratory and oral tract. Several chemicals based antimicrobial substances, like sanitizers and disinfectants are available to break this chain of transmission and prevent the development of disease. But these have some long-lasting side effects in human e.g. making the skin rough and scaly, depletion of beneficial microflora and even contributing to antimicrobial resistance. On the other hand, the use of natural and herbal based products addresses these drawbacks quite effectively. India has been traditionally known to possess a rich source of many plants and herbs, possessing potent antimicrobial, especially antiviral property. Thus, in the present study, the antiviral property of certain natural extracts e.g. beet, orange, drumsticks, cinnamon, ginger, neem and oregano, was evaluated by checking their inhibitory potential against bacteriophages, as bacteriophages are viruses with similar morphological and physiological features. It was observed that among these cinnamon, ginger and oregano exhibited the highest activity against the bacteriophages, which clearly demonstrated their potential to be used as antiviral therapeutics. Further this study also highlighted the need for further exploration of other natural extracts for treatment and prevention of various diseases.

Key words: Natural extracts, viral infections, anti-viral, antimicrobial resistance, chemical sanitizers

1) INTRODUCTION:

As we all know, we are facing viral infections, almost since the start of civilization till date. The viral infection and diseases have been on the rise, leading to more suffering and death all over the world. This is also attributable to the increasing rate of mutation in viruses.

Viruses survive and replicate only in the host, high-jacking the replication machinery of the host for maintenance and self-propagation. Pathogenic viruses, e.g., presently circulating Coronaviruses and its lineage omicron sub-strains, and others like Dengue, HIV, Ebola etc get entry in the human body mainly through oral—respiratory tract for example mouth, nose, eye and sometimes through vectors e.g insects etc. Prevention of viral infection is really

challenging due to its evolved adaptation strategies, metabolic properties and uniquely high rates of mutation. Unfortunately, still there are only very few medicines which can cure viral infections, even the most routine ones causing diseases like the common cold, influenza, chicken pox, etc.

For decreasing the transmission of infection and disease, generally chemical (mainly alcohol) based hand sanitizers and disinfectants are routinely used for killing the microbial pathogens on the surface of the skin and other physical surfaces [3]. Generally, these are quite effective in breaking the chain of transmission and providing good protection against the microbial pathogens present on the surfaces, including [3]. But, on the flip side, these possess many harmful effects also. It principally contains alcohol and other kinds of chemicals. On prolonged use of these, several kinds of dermal complications may develop in the user, e.g., the skin losing its natural texture and color, getting rough and scaly itching, burning effect and eczema etc. Significantly, it might also deplete the beneficial microflora present on the surface of skin. Moreover, with a dangerous implication, it can even contribute to the gradual rise in the antimicrobial resistance of different kinds of pathogens present on the skin surfaces, which later become resistant to other antimicrobial agents e.g. antibiotics. This has been already highlighted in some of the recent studies, reported from across the globe.

Due to this damage and threat posed by the chemical-based substances, there is an urgent need to look for alternative therapies and measures to protect against the infections contracted through skin surfaces, at the same time preserving the natural texture and softness of the skin. There is a huge resource available of these alternative molecules in the nature, mainly in the form of various plant-based products and natural compounds e.g., herbs, fruits, vegetables and spices etc., many of which distinctly possess antimicrobial properties against a wide variety of pathogens. The most significant advantage is that there are no side effects from these natural compounds, unlike the chemical and alcohol-based products.

The reported documentation of the medicinal properties and applications of these natural extracts, is almost as old as the origin of human civilization on earth. From ancient times, these products have been used throughout the world, across different nations, cultures and traditions, and for the prevention and treatment of various kinds of diseases, including those of microbial origin. Of late, the focus on the use of these herbal and natural medicines for therapeutic purposes, because of their validated effects, commonly termed as Naturopathy, Ayurveda, or Holistic medicine, has increased. Besides providing potent antimicrobial effects they also boost the general immunity of the body, which further enhances the defense capability of the host against the pathogens. These herbal extracts can be formulated in the form of medicines to be taken orally (internal use, e.g. as antiviral), or it can be applied topically also (external use, e.g. anti-bacterial) to treat various kinds of microbial diseases and infections. Herbal sanitizers, sprays and disinfectants for topical applications, based on natural products, would not have any deleterious effect on the skin, unlike the synthetic ones. Furthermore, the abundance of the natural extracts in the world, can ensure a steady production and cheaper price of these products. Significantly, it would also not contribute to the development of resistance in the pathogens, unlike their chemical counterparts.

So, it is quite apparent that any natural or herbal antimicrobial substances, formulated either as medicines or meant for topical use, possess many advantages over their synthetic counterparts. But still, only a handful of the different kinds of natural extracts have been utilized till date for the purpose. There are still many other unexplored natural sources available in nature, which can be evaluated for their antiviral and other therapeutic potential. In the future, the ones with promising activity can be formulated into various therapeutic products e.g. natural or herbal based drugs, sanitizers, prophylactic agents etc.

Thus, this study was undertaken to specifically check the antiviral activity of certain unexplored natural extracts against bacteriophages. Bacteriophages, are viruses which infect only bacteria, but possessing unique morphological and physiological properties like any other viruses. The ones demonstrating significant activity, can be potentially further formulated into effective antiviral therapies, both for the treatment and prevention of viral infection in the future.

2) METHODOLOGY:

2.1 Bacteriophage Isolation:

For the isolation of bacteriophages, sewage sample was collected locally, from the same source three times. It was further processed as mentioned below. The protocol was optimized, which included centrifugation of the sewage sample at 5000 rpm for 20 minutes at room temperature. Supernatant was collected and recentrifuged for the same rpm and duration, but this time at 4^{o}C. The pellet was discarded thereafter, and the supernatant was finally collected in a fresh sterilized tube, called the bacteriophage lysate. The bacteriophage lysate was further enriched by mixing with individual bacterial cultures in 1:1 ratio by volume, and then incubating in a shaker at room temperature for one hour. Plating of this mixture over bacterial lawn in agar plates was done by three methods, *viz.*: Direct Inoculation, Molten Agar and Cup Borer method. Based on the efficacy and reproducibility of the plaques observed with the above three methods, Direct Inoculation method was selected for further experiments. Earlier to that, bacterial lawns were prepared on agar plates by pouring three ml culture each of following strains, e.g. *Staphylococcus epidermis, Pseudomonas aeruginosa, Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* over the plate and distributing it uniformly. The extra culture was discarded and the plate was dried at room temperature.

2.2 Selection of extracts:

The particular plant extracts were selected according to their potential antimicrobial especially antiviral effect, demonstrated by literature survey, traditional knowledge and availability in the local markets. Finally, the following extracts, totally seven were used in the study, *viz.* beet, oregano, drumsticks, cinnamon, ginger, neem and orange.

2.3 Determining 'antiviral' effect of the extracts.

For determining the inhibitory properties of the plant extracts against the bacteriophages, the bacteriophage lysate was also similarly mixed (treated) with the individual plant extracts in the ratio of 1:1 by volume. It was further incubated for one hour in an orbital shaker at room temperature and low speed. Mixtures of lysate were also prepared with 1:5 and 1:10 dilution of extracts (to check the effect of the extracts at lower concentrations) and processed similarly as before. After the 'treatment', all the mixtures of lysate and extracts (including the different dilutions) were plated over individual bacterial culture lawns, prepared earlier on agar plates. Among comparison of the plaque activity of the bacteriophage against different bacterial cultures, it was observed that the most promising activity was observed against *P. aeruginosa* The surplus mixture was discarded and plates dried at room temperature. Later these were kept for overnight incubation at room temperature for the observation of plaques.

3 RESULTS

3.1 Bacteriophage isolation

Interestingly, each time the sewage sample was collected (totally 3), it showed plaque formation exhibiting lytic effect, but against a different bacterium. The first time the isolated bacteriophages from the sample, showed prominent plaque formation against the *E.coli*. whereas at the second and third time, it showed significant lytic effect against *Pseudomonas Aeruginosa* and *Staphylococcus epidermis* respectively (Table 1) (Fig 1).

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3.1.2 Antiviral effect on extract

Totally seven extracts *viz.* beet, oregano, drumsticks, cinnamon, ginger, neem and orange were used in the study. The results demonstrated that the bacteriophage lysate treated with extracts of Cinnamon, Ginger, Orange, Beet and Drumstick) produced absolutely no plaques against the bacterial cultures, both in concentrated and diluted forms, indicating towards a significant bacteriophage-inhibitory and possible broader antiviral activity of these extracts (Table 2 and 3) (Fig 2). Though at long durations of incubation (24hr), the lowest concentration (i.e. the highest dilution) of Beet and Drumstick showed the appearance of few plaques (Fig 3). Conversely, the mixture of lysate with extracts of Neem and Oregano, produced 05 and 08 plaques respectively, in the agar plates. These though less in number, pointed to their slightly diminished action against the bacteriophages and probably less potential as antiviral compounds (Table 2 and 3).

Specifically, in case of beet, the concentrated extract showed no plaques, whereas the 1:5 and 1:10 dilutions showed 04 plaques and 07 plaques respectively. Drumstick exhibited marginally better anti-bacteriophage effect than beet. There was no plaque observed both in the concentrated as well as 1:5 dilutions, whereas only 03 plaques were observed with 1:10 dilution. So, it can be also considered almost as effective the other extracts, for inhibiting bacteriophages (Table 3) (Fig 3).

4. Discussion

The present study was undertaken to evaluate the inhibitory potential of natural extracts against bacteriophages. It was observed that among the various extracts used, barring neem and oregano and up to an extent beet, almost all of them e.g. cinnamon, ginger, beet, oregano, drumstick demonstrated significant inhibitory effect against bacteriophags. This in turn, pointed to their possible application in future, as novel antiviral therapeutic molecules. The antiviral potential of Oregano and Neem was found to be comparatively lower than the other extracts.

As discussed above, this study clearly exhibited that certain selected natural extracts, with higher and potent activity against bacteriophages can serve as ideal candidate for developing antiviral therapeutics and prophylactic agents e.g. medicines, hand sanitizers, surface disinfectants, sterilant etc. These findings are also significant for facilitating the discovery of novel drugs against viral diseases, to circumvent their high rates of mutation leading to generation of new variants and even some re-emerging ones. Additionally, these would serve as an excellent alternative for chemical and alcohol-based hand, eliminating the numerous harmful effects the for
development of alternative antiviral therapies in the future. It can alleviate many of the ills inherent in the chemical products, e.g. dermal complications, wiping of the beneficial microflora on the skin and development of antimicrobial resistance among the pathogens, etc.

Similar products have been demonstrated earlier to be very useful in daily life to prevent and treat many diseases, including microbial infections caused by virus, bacteria and fungi (Patankar R.S *et al.,* 2018). Already, many natural and herbal based products with therapeutic potential have been formulated into drugs against various diseases and infections, some of them already running successfully in the market (Stuart B. L. *et al.,* 2002).

5. Conclusion

Plants and herbs usually produce a wide range of bioactive molecules, with diverse medicinal properties and health benefits, e.g., antimicrobial activity, immunomodulatory properties and anti-cancerous effects etc. This study also clearly exhibited the potent and significant anti-viral effects of certain natural products e.g. cinnamon, ginger, beet, orange, drumstick. Because of these inherent health benefits, many other natural extracts have been screened to evaluate their efficacy as medicines for treating other types of diseases. Moreover, as already discussed, these possess less side-effects compared to the synthetic and chemical based products, and even could be also more readily and cheaply available to the people. Therefore, we conclude, based on the findings form this study, that the above-mentioned natural compounds can be formulated into novel and effective antiviral therapeutics. Further this study highlights the scope of similar other natural and herbal products in the future to serve as an exciting alternative for treating and preventing various kinds of disorders. sanitizers and surface disinfectants. Many natural extracts have already been screened till date to evaluate their efficacy for treating various types of viral diseases. Moreover, these can be produced at cheaper rates, readily and in bulk due to their abundance in nature. Thus, it can be said that natural and herbal compounds serve as an exciting resource for the

Bacterial culture name	Bacteriophage Sample 1	Bacteriophage Sample 2	Bacteriophage sample 3
Staphylococcus epidermis	Nil	01 Plaques	543 Plaques
Pseudomonas aeruginosa	Nil	11 Plaques	Nil
Bacillus subtilis	Nil	07 Plaques	Nil
E.coli	Nil	03 Plaques	1 Plaques
Staphylococcus aureus	Nil	03 Plaques	Nil
Salmonella typhi	Nil	10 Plaques	178 Plaques

Table 1: Bacteriolytic activity of the bacteriophages isolated from the same sample collected at 3 different time points, against different bacteria.

Plant extract name	Number of Plaques observed
Cinnamon	No plaque observed
Ginger	No plaque observed
Neem	5 - Plaque
Oregano	8 - Plaque

Table 2: Plaque formation by the bacteriophage lysate post treatment with different plant extract against Pseudomonas aeruginosa.

Plant Extract	Dilution	Number of Plaque
Beet	Pure	No plaque
	1:5	4 plaque
	1:10	4 plaques at 15hrs
		7 plaques observed at 24 hrs
Orange	Pure	No plaques
	1:5	No plaques
	1:10	No plaques
Drumsticks	Pure	No plaques
	1:5	No plaques
	1:10	No plaques at 15 hrs
		3 plaques observed at 24 hrs

Table 3: Plaque formation by the bacteriophage lysate post treatment with different plant extract (both concentrated and diluted form) against *P. aeruginosa*.

A. B. C. D. E.

Fig 1: Bacteriophage lysate activity on A. Staphylococcus epidermis, B. Pseudomonas aeruginosa, C. Bacillus subtilis, D. E.coli, E. Staphylococcus aureus.

Fig 2: Effect of Phage lysate and different extract mixture against pseudomonas aeruginosa.

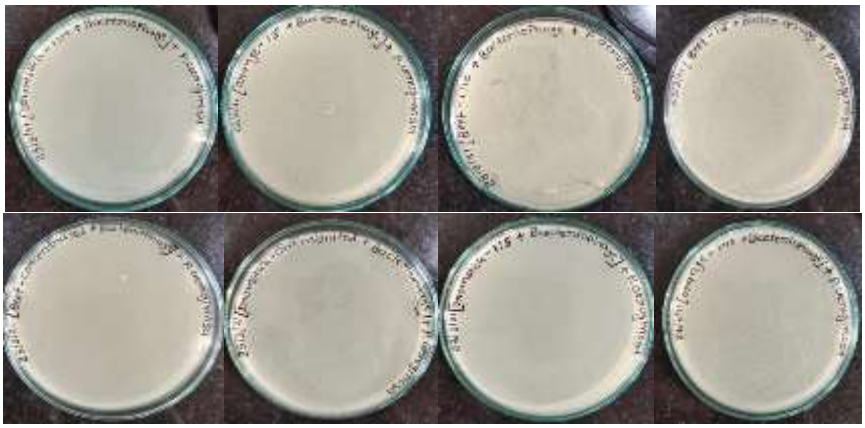

fig 3: Effect of phage lysate and extract activity against *P. aeruginosa* in different dilutions.

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