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ORIGINAL ARTICLE

Evaluation of Antibacterial Properties of Harpeen Against Selected Bacterial Pathogens

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ABSTRACT

The occurrence of antibiotic resistance in bacteria is a serious threat to global health. The dried form of whey is called Harpeen and has antibacterial properties. In this study, the antibacterial properties of Harpeen were evaluated against pathogenic bacterial strains including *Pseudomonas aeruginosa, Escherichia coli, Methicillin-resistant Staphylococcus aureus (MRSA), Proteus, Klebsiella pneumoniae, Streptococcus pyogenes, Streptococcus, Salmonella typhi, Enterobacter, Bacillus subtilis* via agar-well diffusion method. Harpeen exhibited significantly higher antibacterial activity against *Proteus* and *Klebsiella pneumoniae* in comparison to other selected bacterial strains. This natural product can be utilized as a potent antimicrobial agent.

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INTRODUCTION

The lack of effective antibiotics against the evolving bacteria and their alarming resistance towards these antibiotics has been a race against time, long acknowledged as a fact [1, 2]. Microbial infections leading to the deaths of more than half of the population in developing countries have been a major global health security concern for quite some time [3, 4]. Respiratory and skin infections are caused by some *Staphylococcus spp.* and *Streptococcus spp.* Gastrointestinal and urogenital disease is caused by *pseudomonas* and some *Enterobacteriaceae* which are resistant to different antibiotics [5]. The much-known cause of illness and death in a developing country is food poisoning [6]. The most common cause of food poisoning is bacterial contamination, including certain Gram-negative bacteria such as *Salmonella typhi, Escherichia coli, and Pseudomonas aeruginosa* [7].

This precarious situation has brought back the need for affordable, effective, and innovative medicines for the treatment of microbial infections[4]. As bacteria continue to evolve and increase their resistance to the present antibiotics, an immediate need arises to produce new and improved antibiotics [8]. The use of indigenous products for the extraction of antimicrobial compounds has been an alternative [4].

The need for new antibiotics has led to the appearance of synthetic antibiotics made by altering the chemical composition of compounds to match the properties of present antibiotics. This poses new challenges with the compatibility and applicability of these antibiotics when administering them. Although these chemical modifications have proven an efficient method for the development of new effective antibiotics, nevertheless, these modified antibiotics are used against microbial resistance pathogens. This requires the development of antimicrobial drugs with a completely new mode of action. Finding new novel drugs is a challenge in itself. The number of resources, time, and expertise required have a low chance of success [9].

There is an increasing interest in the search for new antimicrobial products obtained from natural sources such as plants, fungi, and bacteria [10]. Microbial and plant products are considered the main sources of novel drug molecules [10]. Some species like mushrooms have been tested to have terpenoids, flavonoids, and alkaloids[11]. They are being researched to obtain further bioactive compounds. Another product honey obtained from honey bees (*Apis mellifera*), has been used to produce extracts that have both clinical and medical uses [12]. They are used for limiting inflammation and promoting the healing process.

Harpeen is a traditional dairy byproduct manufactured locally in Parachinar Kurram Agency, KPK, and Pakistan and nearby areas of Afghanistan regions. Being dairy products, on the other hand, Harpeen comes under the category of natural product derivatives possessing antimicrobial properties [13]. The origin of Harpeen dates back a few centuries as recorded by the locals of the area, a product used by their ancestors. This product is extracted from another traditional product called Lassi (yogurt)-based drink produced by the mixing of yogurt). Lassi is then filtered using a cotton fabric, which produces an extract of whey. The whey is then heated and stirred to become solidified called Harpeen. The harpoon can be consumed orally and used for the treatment of throat infection, coughing, and stomach-ache. It is consumed normally on a need basis. In this study, freshly prepared Harpeen was quantitatively evaluated against bacterial pathogens using the agar-well diffusion method followed by their Minimum Inhibitory Concentration (MIC).

MATERIALS AND METHODS

Bacterial Strains

Pseudomonas aeruginosa, Escherichia coli, Methicillin-resistant Staphylococcus aureus (MRSA), Proteus, Klebsiella pneumoniae, Streptococcus pyogenes, Salmonella typhi, Enterobacter and *Bacillus subtilis* were used in this study and obtained from the Applied Microbiology and Biotechnology Lab of the International Islamic University, Islamabad.

Production of Harpeen

Harpeen is a traditional product which is made from yogurt and is mostly produced in the tribal areas of Pakistan. The preparation of Harpeen consists of the following three steps. separation, heating, and cooling (Figure **1 a-c**).

Separation

Harpeen is a local product that is produced from the dairy product yogurt. The process of Harpeen production is initiated with the continuing stirring of 2 L yogurt for an hour. This led to the production of two products, i.e., Lassi and butter. Butter was discarded while the product of interest, lassi was separated by using different filtration techniques. The total amount of lassi obtained was 1200 mL. In Tribal Areas, often people used fabric materials of varying natures for the filtration of lassi which usually took about a few hours. In the laboratory, the cotton handkerchief was used for the filtration of lassi. The filtered-out material, known as whey, a light-yellow color was collected in a separate beaker. The amount of whey was 800 mL. The remaining material trapped in the cotton handkerchief was cheese which is also an important dairy product.

Heating

The beaker containing 800 mL whey was placed on the magnetic stirrer at 100ºC and heated for 3-4 h to obtain brown precipitates.

Cooling

The sample was cooled down and allowed to solidify. This process takes from a few days to a week to obtain the traditional product Harpeen and its concentration was 18 g.

Figure 1. a) Filtration of lassi, b) heating & stirring of whey, c) Harpeen.

Preparation of Harpeen Extract

Different concentrations of solidified Harpeen (0.5, 1.25, 2.5, and 3.5 g) were added separately in 5 mL of distilled water followed by heating on a magnetic stirrer at 40-50°C for 15-20 min. The prepared extract was poured into falcon tubes and placed at room temperature till further use.

Antibacterial Activity

Approximately 14 g of nutrient agar was dissolved in 500 mL of distilled water and the dissolved mixture was autoclaved at 121°C for approximately 15 min. The medium was poured into sterilized glass plates and allowed to solidify at room temperature. The agar well diffusion method was used to evaluate the antibacterial properties of Harpeen against pathogenic bacterial strains including *Pseudomonas aeruginosa, Escherichia coli, Methicillin-resistant Staphylococcus aureus* (MRSA), *Proteus, Klebsiella pneumoniae, Streptococcus pyogenes, Streptococcus, Salmonella typhi, Enterobacter,* and *Bacillus subtilis*. The diameter of each well was 5 mm. The inoculated plates were incubated overnight in an incubator at 37°C. On the next day, the zone of inhibition was measured with a ruler.

RESULTS

All different concentrations of Harpeen demonstrated significantly higher zones of inhibition against *Proteus, and Klebsiella pneumoniae* as compared to other bacterial strains including *Pseudomonas aeruginosa, Escherichia coli, Methicillin-resistant Staphylococcus aureus* (MRSA), *Streptococcus pyogenes, Streptococcus, Salmonella typhi, Enterobacter,* and *Bacillus subtilis* (Figure **2 A-I**). An overview of zones of inhibition of different concentrations of Harpeen against all selected Gram-positive and Gram-negative bacterial strains is included in Tables **1** and **2**, respectively. In recent studies, honey products have been used against different bacteria like *Staphylococcus aureus and Pseudomonas aeruginosa* both of them are highly resistant to methicillin but more than twenty honey products have been used against them and showed high antibacterial activity against these wound inducing-pathogens. Just like honey, various rough extracts of ethanol and methanol were studied for their activity after the MIC analysis showed the result in between the range of 0.7-0.9 nm. So these natural products are competing against the modern world as compared to the broad spectrum antibiotics[13, 21, 22].

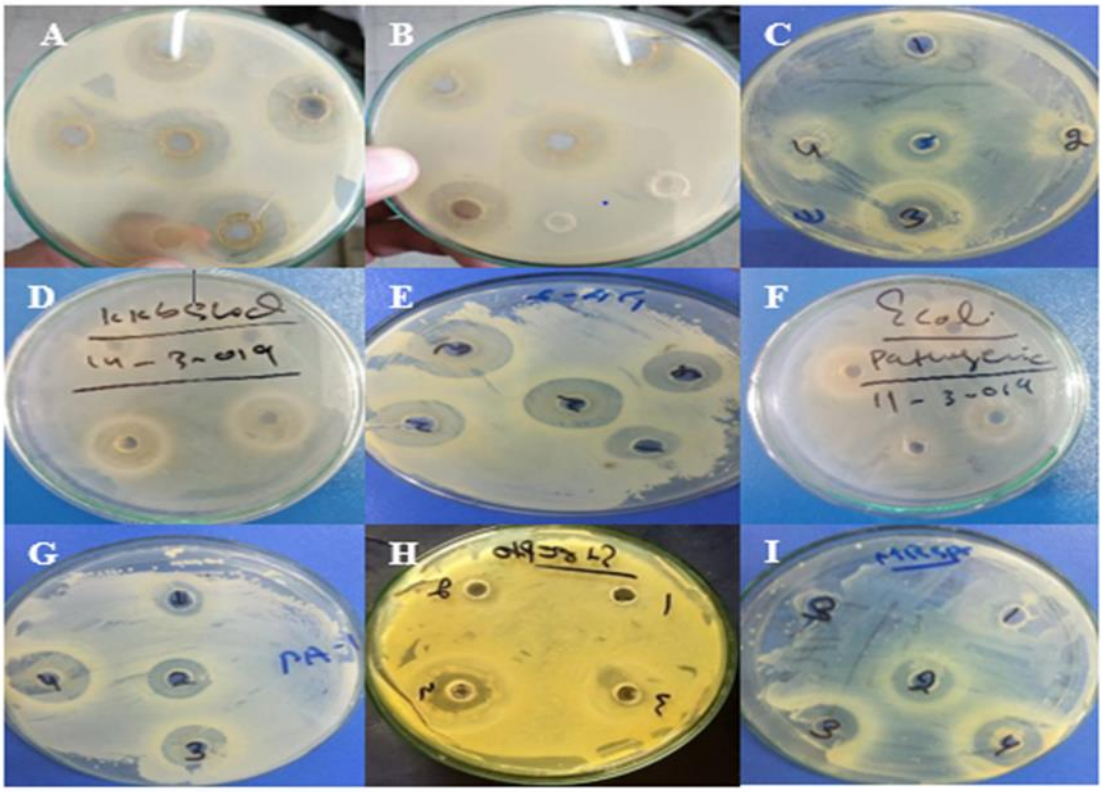

Figure 2. Antibacterial activity of Harpeen against different bacteria *Enterobacter* (A), *B. subtilis* (B), *Proteus* (C), *K. pneumonia* (D), *S. pyogenes* (E), *E. coli* (F), *P. aeruginosa* (G), *S. typhi* (H), *MRSA* (I).

Table 1. An Overview of Zones of Inhibition of Different Concentrations of Harpeen Against All Selected Gram-Positive Bacterial Strains.

Quantity (g/5mL)	*B. subtilis*	*MRSA*	*S. pyogenes*
0.5	3.4±0.18	2.4±0.09	2.4±0.09
1.25	1.7±0.04	1.4±0.03	1.6±0.04
2.5	1.1±0.00	1.1±0.00	1.4±0.03
3.5	0	0	1.4±0.03

Table 2. An Overview of Zones of Inhibition of Different Concentrations of Harpeen Against All Selected Gram-Negative Bacterial Strains.

Quantity (g/5mL)	*Proteus*	*E. coli*	*S. typhi*	*P. aeruginosa*	*Enterobacter*	*K. pneumoniae*
0.5	3.6±0.2	2.6±0.10	1.9±0.07	2.8±0.11	3.7±0.35	3.6±0.2
1.25	2.1±0.05	1.2±0.01	1.7±0.045	1.6±0.043	3.7±0.35	3.4±0.18
2.5	2±0.08	1.1±0.009	1.5±0.04	1.6±0.043	3.6±0.2	3.3±0.18
3.5	1.9±0.06	1.1±0.009	0	1.4±0.03	3.4±0.18	1.8±0.05

DISCUSSION

Antimicrobial resistance of commonly known pathogens is the major cause for the diversion from synthetically produced antibiotics to naturally produced products from various plants and organic sources. Locally various broad-spectrum antibiotics are now known to be diminished from the commercial arena due to this threat put in front by the pathogens and causing severe problems to the general public and scientists to deal with this threat[13, 15, 16].

Since 2014, onwards world has progressively gone towards more organic sources of antibiotics such as various plants and food bioproducts dairy, and fruits. These sources have some reliable compounds that can be extracted and have no side effects at all to be worried about. There are a lot of studies and references about natural compounds having antimicrobial activity against developing diseases in the modern world. The essentials for the production of natural products are hereby present in the source compounds so there is no need for adding artificial agents or helpers for the production of such products [14,17].

Plant extracts are another source of obtaining products for this purpose various plant species like *A. discoridis* leaf extract have a very high value of minimum inhibitory concentration against *S. pneumonia* [15, 18]. Various plants like *Achillea membranacea* have ecologically been used for stomach ailments and as an anti-inflammatory and anti-diarrhea activity [19]. So these plant extracts have various by-products that have been used historically for treating various diseases and now with modern-day technology, these by-products can be extracted from the sources to have some sort of antimicrobial activity against disease-causing pathogens [15, 20].

CONCLUSION

All different concentrations of Harpeen depicted comparatively higher antibacterial activities against *Proteus* and *Klebsiella pneumoniae* as compared to other bacterial strains. Further research should be performed to get the benefit of Harpeen by discovering drugs against microbes.

CONFLICT OF INTEREST

None.

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REFERENCES

1. Theuretzbacher U, Mouton J. Update on antibacterial and antifungal drugs–can we master the resistance crisis? Curr Opin Pharmacol. 2011;11(5):429-32.
2. Walsh TR, Toleman MA. The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. J Antimicrob Chemother. 2011;67(1):1-3.
3. Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, Eloff JN. Antimicrobial activity and cytotoxicity of the ethanol

extract, fractions and eight compounds isolated from Eriosema robustum (Fabaceae). BMC Complement Altern Med. 2013;13(1):289.

4. Srivastava J, Chandra H, Nautiyal AR, Kalra SJ. Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAMs) as an alternative drug line to control infections. 3 Biotech. 2014;4(5):451-60.
5. Neu HC. The crisis in antibiotic resistance. Science. 1992;257(5073):1064-73.
6. Doughari J, Pukuma M, De N. Antibacterial effects of Balanites aegyptiaca L. Drel. and Moringa oleifera Lam. on Salmonella typhi. Afr J Biotechnol. 2007;6(19).
7. Solomakos N, et al. The antimicrobial effect of thyme essential oil, nisin and their combination against Escherichia coli O157: H7 in minced beef during refrigerated storage. Meat Sci. 2008;80(2):159-66.
8. Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003;111(9):1265-73.
9. Fernebro J. Fighting bacterial infections—future treatment options. Drug Resist Updat. 2011;14(2):125-39.
10. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-9.
11. Gebreyohannes G, et al. Determination of Antimicrobial Activity of Extracts of Indigenous Wild Mushrooms against Pathogenic Organisms. Evid Based Complement Alternat Med. 2019;2019.
12. Allen KL, Molan PC, Reid G. A survey of the antibacterial activity of some New Zealand honey. J Pharm Pharmacol. 1991;43(12):817-22.
13. Tonks AJ, et al. Honey stimulates inflammatory cytokine production from monocytes. Cytokine. 2003;21(5):242-7.
14. Mostafa AA, et al. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci. 2018;25(2):361-6.
15. Elisha IL, et al. The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. BMC Complement Altern Med. 2017;17(1):133.
16. Cheesman L, Nair JJ, van Staden J. Antibacterial activity of crinane alkaloids from Boophone disticha (Amaryllidaceae). J Ethnopharmacol. 2012;140(2):405-8.
17. Chariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BPS. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. J Ethnopharmacol. 1999;64(3):265-70.
18. Nguefack J, Somda I, Mortensen CN, Amvam Zollo PH. Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (Oryza sativa L.). Seed Sci Technol. 2005;33(2):397-407.
19. McMaster CA, Plummer KM, Porter IJ, Donald EC. Antimicrobial activity of essential oils and pure oil compounds against soilborne pathogens of vegetables. Australas Plant Pathol. 2013;42:385-92.
20. Duffy B, Schouten A, Raaijmakers JM. Pathogen self-defense: mechanisms to counteract microbial antagonism. Annu Rev Phytopathol. 2003;41(1):501-38.
21. Kluciński W, Dembele K, Kleczkowski M, Sitarska E, Winnicka A, Sikora J. Evaluation of the effect of experimental cow endometritis on bactericidal capability of phagocytizing cells isolated from the blood and uterine lumen. J Vet Med A. 1995;42(1-10):461-6.
22. Woolford MK. The antimicrobial spectra of organic compounds with respect to their potential as hay preservatives. Grass Forage Sci. 1984;39(1):75-9.