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Antibacterial Activity and Mechanism of Action of Crude Aqueous Extracts from Agricultural Waste against Foodborne Pathogenic Bacteria

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ABSTRACT

Foodborne disease causes enormous financial and human losses in the country. Bacteria are the most common causative agent of food poisoning. Controlling the amount of these bacteria in food is thus a critical and urgent issue for public health conservation and preservation. Agricultural wastes, according to various studies, are inexpensive and valuable in large amounts, and are rich in valuable phytochemicals. Thus, this study was aimed at investigating the antibacterial activity of aqueous extracts from several agricultural wastes and their mode of action against foodborne pathogenic bacteria. Aqueous extracts were prepared from the agricultural wastes including, dried wheat straw, barley straw, corn stover, sugar cane bagasse, and peanut peels. The antibacterial activity of the extracts was tested against *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *and Escherichia coli*, through the determination of the minimum inhibitory concentration (MIC) of each extract. The release of lactate dehydrogenase (LDH) and DNA content were evaluated to determine the mechanism of action of the extracts. The results showed the efficacy of the extracts as antibacterial agents with a MIC of less than 1 mg/ml. The extracts largely damage the bacterial membrane; nonetheless, all of the extracts increased the amount of LDH. The change in DNA percentage after extract treatment of the bacterial cultures was negative, indicating that DNA synthesis was inhibited and its degradation was promoted. The findings of this study indicate that the test extracts could be potential food preservative agents against foodborne pathogens after further research is conducted.

Keywords: DNA, Food poising, Food preservatives, Lactate dehydrogenase, Minimum inhibitory concentration.

Introduction

Foodborne diseases are a constant threat. It is estimated that foodborne diseases result in huge financial loss and mortality in the community. Food contamination with chemicals or pathogenic microbes can cause foodborne diseases. Bacteria are the most common cause of food poisoning. The most common foodborne bacteria are *Salmonella, Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Bacillus cereus, Vibrio parahaemolyticus, Campylobacter jejuni,* and *Escherichia coli*.[1] Food safety is one of the top ten priorities of the World Health Organization (WHO). Microorganisms in the food supply cause foodborne diseases, which are a leading source of morbidity and mortality globally. The occurrence of antibiotic resistance among microbes, which is one of the WHO's top ten threats to global public health, worsens the problem. The pathogenesis and treatment resistance of foodborne pathogens is a complex issue that affects a wide range of industries, including agriculture, food animals, natural niches, human populations, the pharmaceutical industry, and clinics.[2] Foodborne disease epidemic warnings must be monitored, reported, and disseminated as soon as possible.

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Disease monitoring systems have been created by national-level agencies such as the Centers for Disease Control and Prevention (CDC) in the United States and many other countries throughout the world to save public health. In the United States, foodborne infections cause around 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths each year. Food gastroenteritis causes 5.4 million infections, 14,700 hospitalizations, and 76 deaths in Australia each year. Various reports have been released for other industrialized countries, including England, Wales, Canada, the Netherlands, France, and New Zealand, which monitor and study the impact of eating food contaminated with germs, toxins, or chemicals.[3] As a result, limiting the prevalence of these bacteria in food is a critical and pressing concern for public health. This, in general, is achieved through chemical preservatives. These chemical preservatives have been considered unsafe for human health. Furthermore, the growth of bacteria resistant to commonly used antibiotic compounds is another problem associated with the use of chemical synthetic preservatives.[4] Because synthetic preservatives have been linked to health hazards, and increased consumer awareness of food safety has resulted in the use of natural bioactive compounds in food preservation. In this regard, recent studies have established the usefulness of plant extracts and isolated components as natural antibacterial agents in the food industry.[5,6] There is an increase in the rate of generating agricultural waste as a result of the fast-increasing population. If these agricultural wastes are not properly handled, it will lead to a serious environmental problem. Environmental pollution is caused by the production of greenhouse gases such as methane and carbon dioxide as a result of improper waste management. Many investigations have found that these agro wastes are rich in active chemicals that can be employed for human benefit.[7] Thus, these wastes can be exploited to develop novel antimicrobial agents.[8] Converting these wastes into valuable resources not only helps to

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create new important compounds, but it also helps to reduce pollution and waste management problems.[9]

This study was therefore conducted to investigate the antibacterial activity of aqueous extracts from some agricultural wastes and their mode of action against some food poisoning bacteria.

Materials and Methods

Source of foodborne pathogenic bacteria

The bacteria employed in the study were *Salmonella enterica* Typhimurium (MTCC 98), *Staphylococcus aureus* (MTCC 87), *Listeria monocytogenes* (MTCC 1143), *Clostridium perfringens* (MTCC 450), *Bacillus cereus* (MTCC 1272), *Vibrio parahaemolyticus* (MTCC 451T), and *Escherichia coli* (MTCC 723). They were purchased from the Microbial Type Culture Collection Center, Chandigarh, India.

Source of agricultural waste

The agricultural wastes including dried wheat straw, barley straw, corn stover, sugar cane bagasse, and peanut peels were collected from farmers in the Al Sharkia region in Egypt. The dried wastes were ground to a fine powder. The practical part of the present study was in the period from January 2021 to January 2022.

Preparation of aqueous extracts from the agricultural waste

The dried agricultural waste powder was extracted in distilled water according to Rocha-Filho *et al*.[10] Distilled water was added to the waste powder in the proportion of 5% (w/v). The mixture was shaken at room temperature for 48 hours before being filtered. The dry extract was produced after drying the filtrates with a rotary evaporator.

Determination of the minimum inhibitory concentration of the waste extracts

The minimum inhibitory concentration (MIC) of each crude aqueous extract was evaluated to determine the antibacterial activity of the test extracts by the conventional broth dilution assay against all the test foodborne pathogenic bacteria. Brain heart infusion broth was prepared with serial dilution of the test extract in test tubes (10 ml each). Each tube received 100 µL inoculum (1×10^6 CFU/mL) of the test bacterial species. The inoculated test tubes were incubated for 24 h at 37°C. After incubation, the turbidity in the sample, and the control (bacterial cultures without extract treatment to monitor the reduction in the turbidity caused by the extract treatment) tubes were measured spectrophotometrically at OD_{600}. MIC was determined as the lowest concentration that causes a decrease in optical density.[11]

Determination of cell membrane integrity

Lactic dehydrogenase (LDH) activity was determined as an indicator of the cell membrane integrity as described by Kalaivani *et al*.[12] The extract was added to the bacterial suspensions to a final concentration of 2 × MIC and incubated at 37◦C for 6 hours. After incubation, the supernatant was collected by centrifugation.

The LDH concentration in the supernatant was determined spectrophotometrically using an LDH release Kit (Nanjing, China).

Determination of changes in DNA content

Bacterial suspensions having an OD_{600} nm of 2.0 were treated with extracts (MIC) for 6 hours at 37°C, then centrifuged at 10,000 g for 5 minutes. After centrifugation, the bacterial cells were recovered as a precipitate. Bacterial genomic DNA extraction kits (Tian Gen Bintech Co., Ltd., Beijing) were used to determine the change in intracellular DNA content.

$$\text{The change DNA content (\%)} = \frac{\text{Final DNA content} - \text{Initial DNA content}}{\text{Initial DNA Content}} \times 100$$

Statistical analysis

The data were presented as the mean of three replicates ± standard error (SE). Analysis of variance (ANOVA) was used to examine statistical differences in antibacterial activity across the different extracts using the SPSS software (SPSS 17.0 for Windows) at P value = 0.05.[13]

Results and Discussion

Antimicrobial agents are required in the food industry to ensure the supply of high quality food. The limitations of artificial preservatives raise the necessity for finding safe and efficient natural antimicrobial agents. Agricultural waste is well-known for its high concentration of bioactive chemicals. As a result, it has the potential to be introduced as a promising source of natural antimicrobials. The use of agricultural wastes as a source of antimicrobial agents serves a dual purpose in that it provides natural, effective, and safe alternatives to synthetic preservatives in the food industry. Furthermore, it eliminates by-products, reducing the negative environmental impact of these wastes.[14] The results of the present study showed the effectiveness of the wheat straw, barley straw, corn stover, sugar cane bagasse, and peanut peel extracts as antibacterial agents, with the MIC of all extracts being less than 1 mg/ml (Table 1). This observation agrees with Makuwa and Serepa-Dlamini, who found that when the MIC of a crude extract is less than 1 mg/mL, it is classified as an antibacterial agent.[15] Similarly, Alzandi and colleagues reported that crude plant extract with a MIC of less than 1 mg/ml is an effective antibacterial agent.[16] The antibacterial activity of agricultural wastes differed significantly, with peanut peels having the highest antibacterial activity, followed by wheat straw extracts, sugar cane bagasse, and maize stover, having the lowest antibacterial activity (Table 1). The difference in antibacterial activity between the extracts is related to the content of the active components; the more active components in the extract, the higher the antibacterial activity.[17] Similarly, Alzandi *et al*.,[16] attributed the difference in the antibacterial activity of the plant extracts to the difference in their chemical composition.

Table 1: The minimum inhibitory concentration (µg/ml) of aqueous extracts from agricultural waste against bacterial species

Treatment Bacterial species	Sugar Cane Bagasse	Wheat Straw	Corn Stover	Peanut Peels
Salmonella enterica Typhimurium	66.345 ± 1.281[a]	45.876 ± 1.280[b]	76.923 ± 1.003[c]	29.372 ± 0.927[d]
Staphylococcus aureus	47.281 ± 1.227[b]	31.295 ± 1.110[d]	66.094 ± 0.983[a]	15.395 ± 1.093[e]
Listeria monocytogenes	43.918 ± 2.093[b]	30.292 ± 1.029[d]	67.297 ± 1.290[a]	12.380 ± 0.536[e]
Clostridium perfringens	38.621 ± 1.029[b]	29.781 ± 1.367[d]	60.253 ± 1.637[a]	14.294 ± 1.289[e]
Bacillus cereus	40.296 ± 1.007[b]	31.907 ± 1.119[d]	63.898 ± 1.378[a]	15.002 ± 0.637[e]
Vibrio parahaemolyticus	64.871 ± 1.782[a]	44.099 ± 1.389[b]	75.291 ± 1.750[c]	31.575 ± 1.290[d]
Escherichia coli	62.179 ± 1.555[a]	39.986 ± 1.067[b]	77.000 ± 2.009[c]	30.402 ± 0.562[d]

Values are means of three replicates ± standard errors. Values followed by different letters are significantly different from each other according to the ANOVA test P value = 0.05

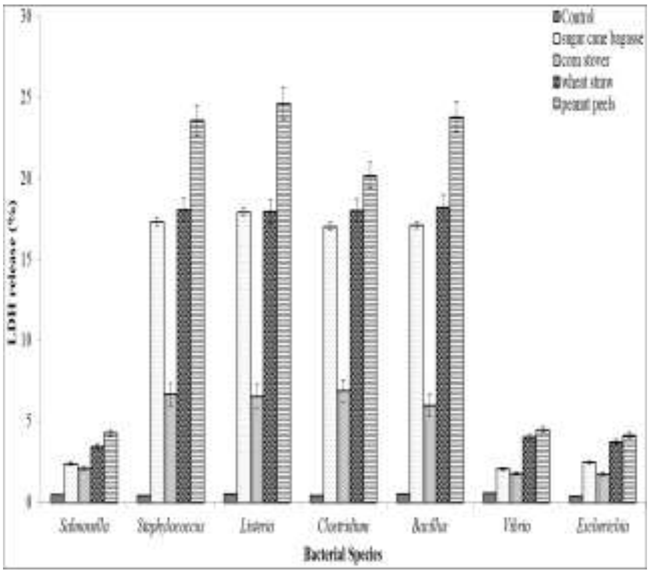

Figure 1: Effect of agricultural waste extracts on lactate dehydrogenase release in different bacterial species. Column values are the means of three replicates. The bars represent the standard errors.

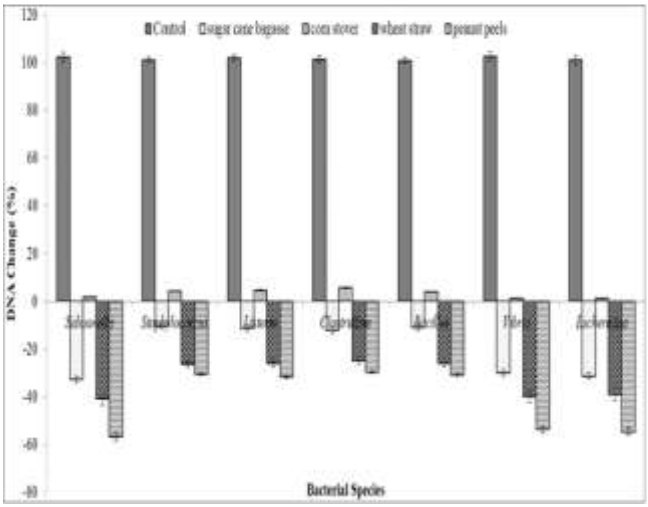

Figure 2: Effect of agricultural waste extracts on change in DNA content in different bacterial species. Column values are the means of three replicates. The bars represent the standard errors.

Agricultural wastes are lignocellulosic biomass, which mainly consists of cellulose, hemicellulose, and lignin, which are considered a rich source of sugars and phenolic compounds. Phenolic compounds can reduce food rancidity by preventing the formation of volatile molecules due to their capacity to inhibit lipid peroxidation. The antioxidant, anticancer, anti-inflammatory, and antibacterial properties of phenolic acids and their derivatives are well documented.[18]

The antibacterial activity of the same extract significantly differed among the different bacterial species. The extracts were more effective on the gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens,* and *Bacillus cereus*) than on the gram-negative bacteria (*Salmonella enterica* Typhimurium, *Vibrio parahaemolyticus,* and *Escherichia coli*) as presented in Table 1. The difference between the gram-negative and gram-positive bacteria in their response to antibacterial agents is related to the structure of their cell membranes. Gram-negative bacteria have an outer layer (outer membrane) consisting of inner phospholipids and outer lipopolysaccharides with the presence of proteins such as porins and others that control the movement of materials across the membrane. This layer is not found in gram-positive bacteria. Any alteration in the outer membrane is considered a signal to the gram-negative bacteria, allowing them to develop antibiotic resistance reactions against the antibacterial agents.[19] As shown in Figure 1, the gram-negative bacteria cell membrane is less affected by antibiotic action than the gram-positive bacteria cell membrane. The release of lactate dehydrogenase (LDH) in the gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens,* and *Bacillus cereus*) was higher than that in the gram-negative bacteria (*Salmonella enterica* Typhimurium, *Vibrio parahaemolyticus,* and *Escherichia coli*). Cellular breakdown and loss of cell membrane activity are considered indicators of cellular breakdown and LDH is released into the surrounding medium by an intracellular enzyme.[20] Antibacterial agents can affect the bacterial cell membrane by causing changes in the phospholipid layer, which causes structural disorders in the membrane. The decrease in permeability and the release of cell components are caused by the disintegration of the external membrane.[17] Another mechanism of action for antibacterial agents is to prevent the synthesis and degradation of nucleic acids.

The results in Figure 2 showed that agricultural waste extracts reduced the DNA content of bacteria, notably gram-negative bacteria. The negative charge in the percent change in DNA content not only indicates that new DNA synthesis is inhibited but also ensures its destruction.[21] Antibacterial agents primarily affect bacterial DNA by inhibiting the DNA gyrase enzyme, which is responsible for the activation of bacterial DNA supercoiling and uncoiling, as well as DNA replication.[5] The results show that crude extracts of wheat straw, barley straw, maize stover, sugar cane bagasse, and peanut peels are highly effective as antibacterial agents against foodborne bacteria *in vitro*. More research is needed to determine which components of these wastes are most beneficial in giving them antibacterial action. Also, to determine if these agricultural wastes may be utilized as food preservatives, they should be tested on real food samples.

Conclusion

The findings of this study revealed that aqueous extracts from agricultural wastes are effective as antibacterial agents with a MIC of less than 1 mg/mL. The extracts largely damage the bacterial membrane; nevertheless, all of the extracts increased the quantity of LDH. The percentage change in DNA after extract treatment of the bacterial cultures was negative, indicating that the extracts resulted in inhibition of DNA synthesis and enhanced its degradation. The test extracts could be used as food preservatives against foodborne pathogens after more investigation of real food samples.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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