Antibacterial Efficacy of Medicinal Plant Extracts against Food Associated Bacteria

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Abstract

Background: The growing concern of antibiotic resistance and the pressure for safer methods of food preservation renew interest in the antibacterial properties of medicinal plants. This paper presents a screening exercise carried out on the antibacterial activity of 9 selected medicinal plants against food associated bacteria namely, Bacillus cereus and Escherichia coli. The potential of these plants to act as natural antibacterial agents was compared with the efficacy of chemical preservatives and antibiotics. In this context, an attempt was made to extract the phytochemical composition through the most potential extracts. **Result:** The agar well diffusion method was carried out to test the antibacterial efficacy in extracts of Jamun, Black Pepper, Marigold, Bel Patra, Saunth, Sauf, Cinnamon, Timur, and Mulethi. Antibacterial activity of various extracts against E. coli and B.cereus: Only the extracts of Jamun and Cinnamon showed very high inhibitory activities against both E. coli and B. cereus whereas there were no inhibition zones in case of the chemical preservatives like acetic acid and benzoic acid. Gentamycin showed maximum inhibitory activity against both test bacteria followed by chloramphenicol and erythromycin. Bacillus cereus was sensitive against all tested antibiotics. Minimum Inhibitory Concentration of ethanolic extract of Jamunwas estimated to be 2.5% (w/v) i.e. 25mg/ml against E. coli and 1.25% (w/v) i.e. 12.5mg/ml against B. cereus.The combination of ethanolic extract Jamun and Cinnamon extracts againstE. coli showed synergistic activity with a zone of inhibition of 22 mm. **Conclusion:** The phytochemical analysis showed the presence of alkaloids, saponins, phytosterols, triterpenes, phenols, flavonoids in Jamun ethanolic extract and absence of carbohydrates, proteins, and amino acid. The findings suggest that extracts of Jamun and Cinnamon could work as natural preservatives against chemicals, opening some promising avenues for enhancing food safety and extending shelf life by inhibiting harmful bacteria.

Keywords: antibacterial, food associated, E. coli, B. cereus, antibiotic, chemical preservatives

Introduction

Food poisoning is a serious health threat caused by organisms such as bacteria, viruses, and parasites that can contaminate food at any stage of production. The majority of cases are linked to bacterial contamination during food preparation, prompting researchers to explore safer agents for use as food preservatives. Globally, microbial food spoilage remains a significant issue, leading to considerable food waste and loss, even in developed countries. Estimates suggest that up to 40% of global food is lost annually due to various factors, including microbial spoilage. Bacteria, yeast, and molds are the primary microorganisms responsible for the deterioration of numerous food products (Lianouet al., 2016). Once these microorganisms contaminate food, they thrive on the nutrients, producing metabolites that lead to spoilage. Additionally, foodborne diseases resulting from the consumption of contaminated food products continue to pose a significant public health concern(Kirk et al., 2017).

Microorganisms are said to be part of the environment, and therefore, there is a clear possibility of contamination at any stage, be it harvesting, slaughtering, processing, or even during the packaging process.Some microorganisms may survive adverse conditions usually applied to food preservation—low temperatures, modified atmosphere and vacuum packaging, while others are even resistant to conventional pasteurization (Elina et al., 2017).Knowing the types of bacteria causing spoilage and the required conditions of each is very important in the development of efficient methods of food preservation. Bacillus cereus and Escherichia coli are the species causing the spoiling of some food products, which are explained here with the conditions required by these bacteria.Bacillus cereus belongs to environmental Gram-positive, motile, spore-forming, rod-shaped bacteria. Recently, B. cereus has been accepted as a cause for severe and occasionally lethal, not gastrointestinal tract infections. Intestinal or non-intestinal pathogenicity by B. cereus is due to the production of tissue-destructive exoenzymes (Bottone, 2010). There are two basic types of food borne disease by B. cereus: one is the emetic toxin-induced disease, which results in vomiting; the other causes diarrheal disease through the action of enterotoxin(s) (Ehling - Schulz et al., 2019).Escherichia colibelong to the family Enterobacteriaceae. These pathogenic strains relate to food and water-borne gastroenteritis or extra-intestinal diseases. This is a facultatively anaerobic, Gram-negative rod bacterium that ferments lactose. The pathogenic E. coliis considered enteropathogenic, enterotoxigenic, enter invasive and enterohemorrhagic. The enterotoxigenic E. colisecrets enterotoxins, leading to diarrhea. Humans are the chief reservoirs (Smith et al., 2009;Hassan and Frank, 2011).

Consequently, there is a growing concern among consumers about the health risks that synthetic additives can cause, leading to a decrease in the trends of their application for food preservation (Kalem et al., 2017).One substitute to control microbial activity is the

application of synthetic antibacterial agents as preservatives. However, their repeated use has accumulated chemical residues in the food supply and initiated microbial resistance to synthetic antibiotics(Akinyemi etal., 2006).This finally opens up an emerging need for eco-friendly strategies that would definitely be of help in the inhibition of pathogenic bacteria, therefore, promoting a longer shelf life of food products without chemical preservatives(Clarke et al., 2017).

In light of this, there have been various studies focusing on the possible application of plant extracts as natural preservatives. According to Acharjeeet al., (2022) for a long time, people have used crude extracts of different parts such as roots, stems, flowers, fruits, and twigs of medicinal plants for the treatment of various human diseases. These plants are rich in flavonoids, alkaloids, tannins, and terpenoids, phytochemicals that have antibacterial and antioxidant properties (Ali et al., 2023). It is due to these concerns; there has been increased focus of research toward developing an effective, safer, and natural food preservative. It has special value in this context: using plant extracts as antibacterial agents for food preservation, on the basis of their bioactive compounds that treat illnesses and inhibit the growth of bacteria. Understanding the mechanisms of the antibacterial effect of extracts from medicinal plants is very key to their optimal use as natural shelf-life extending and quality-preserving agents. Keeping in view the above facts the screening of antibacterial activity of medicinal plant extracts against food associated bacteria and phytochemical analysis of the best plant extract was done.

Materials and Methods

Procurement of Culture

Bacterial cultures were procured from the Department of Biosciences, Chandigarh University. For preliminary checking for purity and specificity, Gram staining was used for initial verification. Cultures were propagated under aseptic conditions by streaking a loopful of the stock onto the surface of nutrient agar plates, which were then incubated at 37°C for 24 hours to allow for growth.

Solvent extraction

Medicinal plant materials were obtained from the local market of Mohali, Punjab and different regions of Himachal Pradesh. The procured materials were properly washed with tap water to remove impurities in the material. They were dried in a hot air over at 62°C for 24 hours to ensure constant moisture content. After drying, the materials were ground into a fine powder using a grinder and made ready for extraction.20% (w/v) of the plant extract was prepared in different solvents such as distilled water, ethanol and petroleum ether. The powder was mixed with solvents and kept at room temperature for 24hrs. The extract was

filtered using the Whatman filter paper and the filtered extracts were stored in the refrigerator for the further experiment (Manilal et al., 2023).

Preparation of Chemical preservatives stock

For antibacterial activity, the solutions of acetic and benzoic acid were prepared at a final concentration of 1% (v/v) in distilled water. Sterilization on these solutions was conducted by Millipore filter and then used in association with the agar well diffusion method.

Antibacterial activity of Plant extracts and Chemical preservatives by Agar well diffusion method

Antibacterial activity of Plant Extracts and Chemical Preservatives were screened using agar well diffusion (Gonelimaliet al., 2018).Sterile nutrient agar plates were prepared. 100µl of bacterial suspension (1.5x 10^6Cells per ml) was spread in sterilized NA plates using sterile cotton swabs. 6mm wells were punched on agar plates using sterilized borer. 50-100 microliter of each plant extract and chemical food preservative was poured in the wells. Plates were incubated at 37°C for 24 hours. Solvents were poured in wells used a negative control. Zone of inhibition was observed and diameters of ZOI were measured using transparent ruler meter rule in mm.

Antibiotic susceptibility pattern of test bacteria

Antibiotic susceptibility pattern test bacteria were done by using Kirby-Bauer disc diffusion method. In this method, 100microliter of both the bacterial suspension was spread on sterilized NA plates using sterile cotton swabs. Antibiotic disc was placed on the surface of agar plates having bacterial culture suspension. The plates were incubated for 24 hours. After 24 hours, ZOI was observed and diameter of ZOI was recorded in mm (Saif et al., 2017).

Minimum inhibitory concentration (MIC) determination of ethanolic extract of Jamun leaves

The Minimum inhibitory concentration of the most potent plant extract was determined by macrodilution agar plate technique. Extracts were made in ethanol at concentrations of 0.2g/ml, 0.1g/ml, 0.05g/ml, 0.025g/ml, 0.0125g/ml, 0.00625g/ml, and each concentration was sterilized by Millipore filter. The plates were incubated at 35°C for 24 hours, and the MIC was defined as the lowest concentration of the extract where a clear zone of inhibition is produced(Sukeriet al., 2021).

Synergistic activity of ethanolic extracts of Jamun and Cinnamon

In the current study, the synergistic antibacterial activity of ethanolic extracts of Jamun and Cinnamon were evaluated by combining them at a 20% concentration each. The spread was done on agar plates inoculated by the cultures of bacteria, incubated at 37°C for 24 hours, followed by measurements of diameters of inhibition zones to assess if combined extracts demonstrated enhanced antibacterial potency compared to individual extracts (Dutta et al., 2021).

Phytochemical Analysis

Phytochemical analysis was carried out to determine the presence of phytochemicals in the most potent plant extract, Jamun leaves ethanol extract. The various methods were used to analyzedifferent phytochemical constituents (alkaloids, carbohydrate, saponins, phytosterols, triterpene, phenolic compounds, flavonoid, proteins and amino acids) (Ramos et al., 2017).Hager's reagent was added to filtrates. The formation of yellow precipitates indicates the presence of alkaloids.The filtrates were heated with Benedict's reagent. An orange-red precipitate indicates the presence of reducing sugars.Extracts were diluted with distilled water and shaken. The formation of a 1 cm layer of foam indicated the presence of saponins. The extracts were treated with chloroform, acetic anhydride, and strong sulfuric acid. A brown ring at the junction indicated the presence of phytosterols.The extracts were treated with chloroform and strong sulfuric acid. A golden yellow color indicated the presence of triterpenes. Ferric chloride solution mix with test extracts. A blue-black color indicated the presence of phenols. In lead acetate test, addition of lead acetate to the extracts. Formation of yellow precipitates indicated presence of flavonoids.Xanthoproteic test involves treatment of the extracts with concentrated nitric acid. A yellow color development indicated the presence of proteins. In ninhydrin test, where the extracts boil with Ninhydrin reagent. Development of a blue color indicated the presence of amino acids.

Results

Antibacterial activity of Plant extracts by Agar well diffusion

Antibacterial susceptibility of nine plant extracts to Escherichia coli and Bacillus cereus was tested by the agar well diffusion method using the ethanol, petroleum ether, and water solvents.

The maximum activity of the ethanolic extract of Jamun was observed in the case of E. coli (23 mm) and B. cereus (17 mm). It was also moderately active against the isolated microorganism

B.cereus in water (13 mm) and inactive in petroleum ether. Cinnamon also exhibited high activity and had the most activity in ethanol, producing an inhibition zone of 22 mm

against B.cereus. It showed some moderate activity against E. coli at 11 mm in petroleum ether, with no activity in water. Black Pepper showed significant activity in ethanol, with 11 mm of inhibition against E. coli and 17 mm against B.cereus. It was inactive in both petroleum ether and water.

The other extracts like Timur, Saunth, Sauf, Mulethi were also active with the zone of activities coming up in ethanol, mainly with Sauf exhibiting a good 21 mm zone against E. coli. In contrast, Marigold and Bel Patra showed no antibacterial activity in any solvent. Overall, Jamun in ethanol emerged as the most effective antibacterial agent, followed by Cinnamon and Black Pepper, highlighting their potential for developing natural antibacterial agents. The results are shown in Table-I, Figure- 1 and 2).

Table I. Antibacterial activity of Plant extracts against E. coli and B. cereus by Agar well diffusion method

S. No.	Plant Extract		Zone of inhibition in mm					
			E. coli			B. cereus		
		Plant Part	Ethanol	Petroleum ether	Water	Ethanol	Petroleum ether	Water
1	**Jamun (Syzygium cumini)**	Leaves	23	ND	NA	17	ND	13
2	**Black Pepper (Piper nigrum)**	Unripe fruit	11	ND	NA	17	ND	NA
3	**Marigold (Calendulaofficinalis)**	Flowers	NA	NA	NA	NA	NA	NA
4	**Bel Patra (Aegle marmelos)**	Leaves	NA	NA	NA	NA	NA	NA
5	**Cinnamon (Cinnamomum verum)**	Bark	NA	11	NA	22	15	NA
6	**Timur (Zanthoxylum**	Bark	17	NA	NA	18	NA	NA

	armatum)							
7	**Saunth (Zingiber officinale)**	Rhizome	14	NA	ND	NA	16	ND
8	**Sauf (Foeniculum vulgare)**	Seeds	21	NA	ND	NA	NA	ND
9	**Mulethi (Glycyrrhiza glabra)**	Roots	17	NA	18	NA	NA	NA

NA- No Activity; ND- Not Determined

Figure1. Antibacterial activity of Plant extracts againstE.coliby Agar well diffusion method- (a) Jamun and Black Pepper(b) Timur and Cinnamon (c)Sauf and Saunth(d) Mulethi(e)Marigold (f) Bel Patra
(E-Ethanol; P.E.- Petroleum Ether; D.W.- Distilled Water)

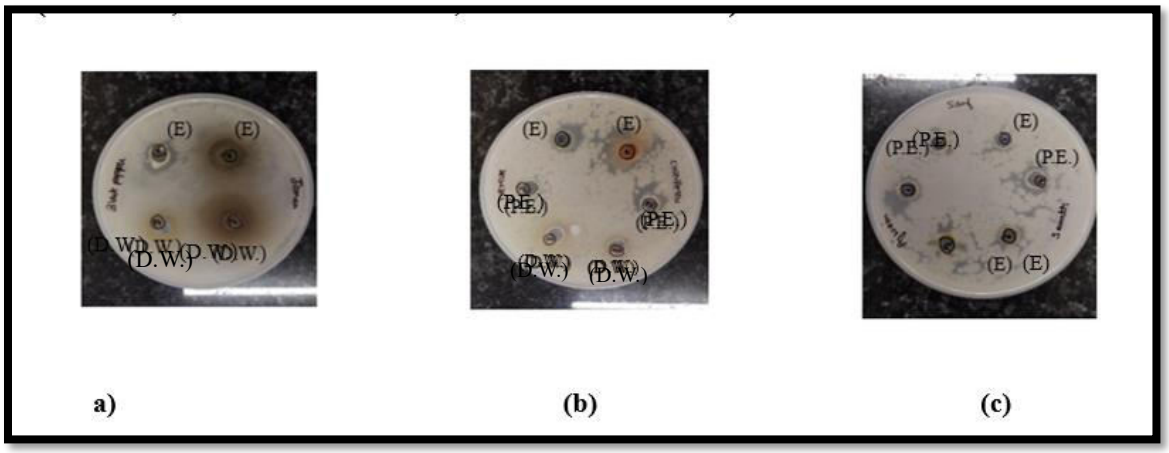
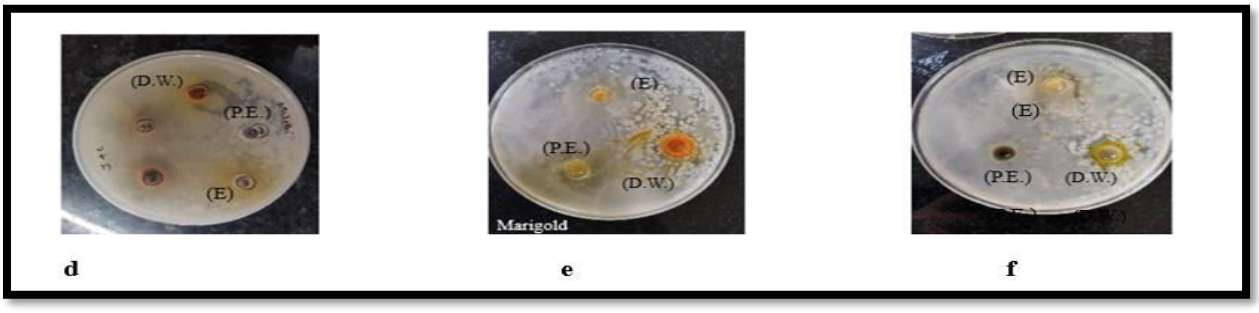


Figure 2. Antibacterial activity of Plant extractsagainstB. cereus by Agar well diffusion method- (a)Jamun and Black Pepper (b) Timur and Cinnamon (c)Sauf and Saunth (d)Mulethi(e) Marigold (f) Bel Patra
(E- Ethanol; P.E.- Petroleum Ether; D.W.- Distilled Water)



Antibacterial activity of chemical preservatives

The two chemical preservatives, acetic acid and benzoic acid, showed no activity against both test bacteria by agar-well diffusion method (Figure- 3).

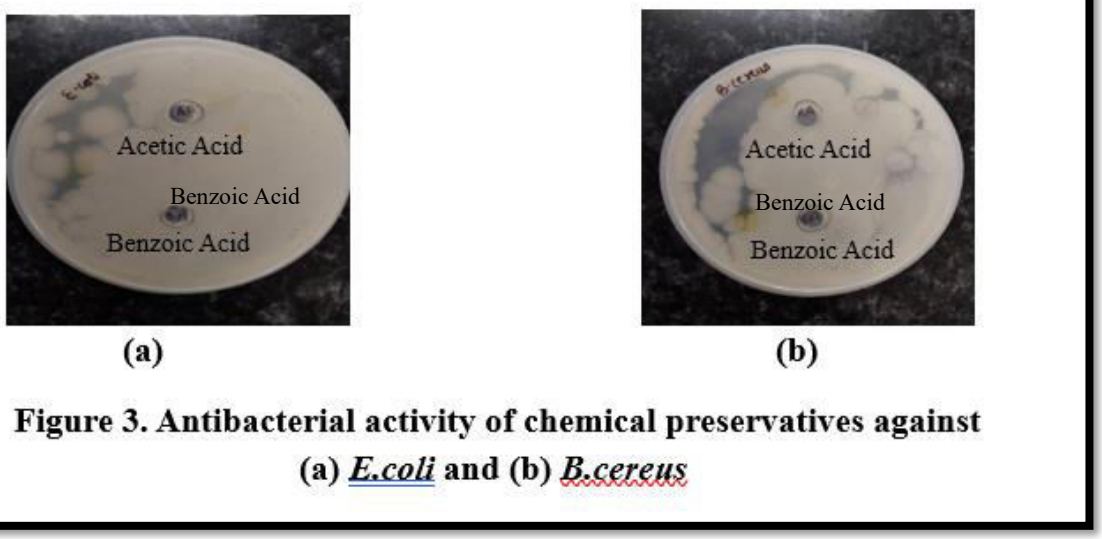


Figure 3. Antibacterial activity of chemical preservatives against (a) *E.coli* and (b) *B.cereus*

Antibiotic susceptibility pattern of Test bacteria

Gentamycin showed maximum inhibitory activity against both test bacteria followed by chloramphenicol and erythromycin. Bacillus cereus was found to be sensitive against all tested antibiotics as shown in (Table- II and Figure- 4)

Table II. Zone of inhibition exhibited by antibiotics against E. coli and B. cereus

Zone of inhibition in mm								
Antibiotics / Test Bacteria	**AMP**	**VA**	**OX**	**GEN**	**E**	**CD**	**C**	**CEP**
E.coli	R	4	R	24	12	R	20	R
B.cereus	25	25	25	25	25	25	18	12

R: Resistance; AMP: Ampicillin; VA:Vancomycin;OX: Oxacillin; GEN: Gentamicin; E:Erthyromicin; CD: Clindamycin: C: Chloramphenicol and CEP: Cephalexin

Figure 4. Antibiotic susceptibility pattern of (a) E.coli and (b) B.cereus

Figure 4. Antibiotic susceptibility pattern of (a) *E. coli* and (b) *B. cereus*

Minimum Inhibitory Concentration of Jamun leaves plant extract

The minimum inhibitory concentration of the Jamun leaves ethanol extract against Bacillus cereus was found to be 1.25% (w/v) and againstEscherichia coli was found to be 2.5% (w/v) (Table- III and Figure- 5)

Table III. Minimum Inhibitory Concentration of Jamun leaves plant extract

Test Bacteria	Concentrations of Jamun ethanolic extract					
	20%	10%	5%	2.5%	1.25%	0.625%
E. coli	26	17.5	15	12	No Zone	No Zone
B.cereus	22	20	17	15	11	No Zone

Figure 5. Minimum Inhibitory Concentration of Jamun ethanolic extractagainst (a) E. coli and (b) B. cereus

Figure 5. Minimum Inhibitory Concentration of Jamun ethanolic extract against (a) *E. coli* and (b) *B. cereus*

Antibacterial activity of Synergistic effect of Jamun and Cinnamon plant extracts

On the basis of maximum zone of inhibition of plant extracts, ethanolic and distilled water extracts of jamun leavesand cinnamon bark were selected to determine their synergistic activity, Hence, the result showed a synergistic effect only against E. coli and did not cause inhibition zones in any other conditions. (Table- IV and Figure- 6)

Table IV. Antibacterial activity of Synergistic effect of Jamun and Cinnamon plant extracts

Bacteria Isolates	**Jamun and Cinnamon [Ethanolic extract]**	
	Zone of Inhibition	
	Ethanol	**Distilled Water**
E. coli	22mm	No Zone
B. cereus	No Zone	No Zone

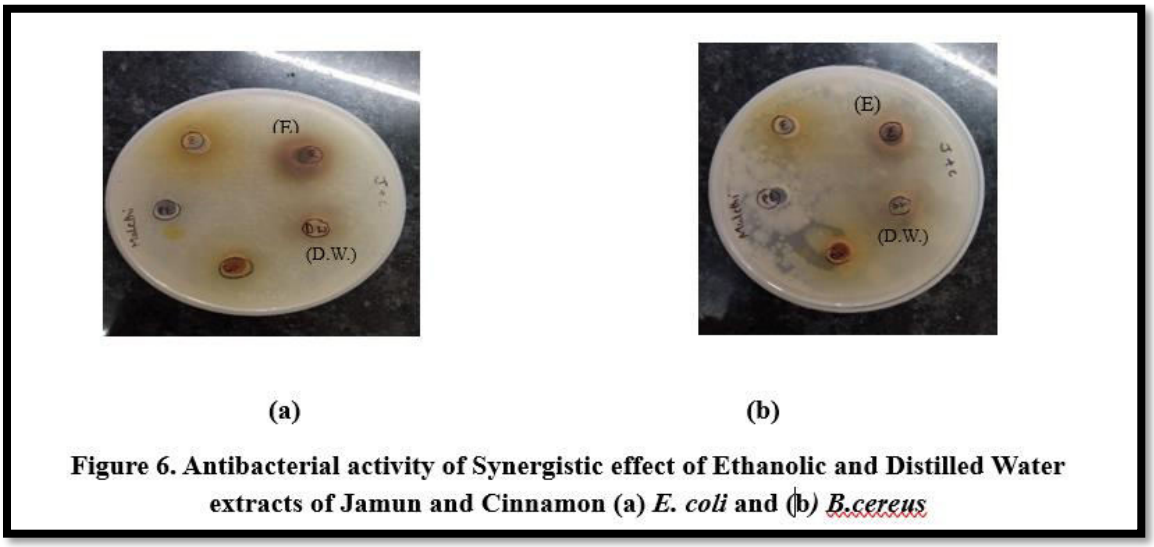
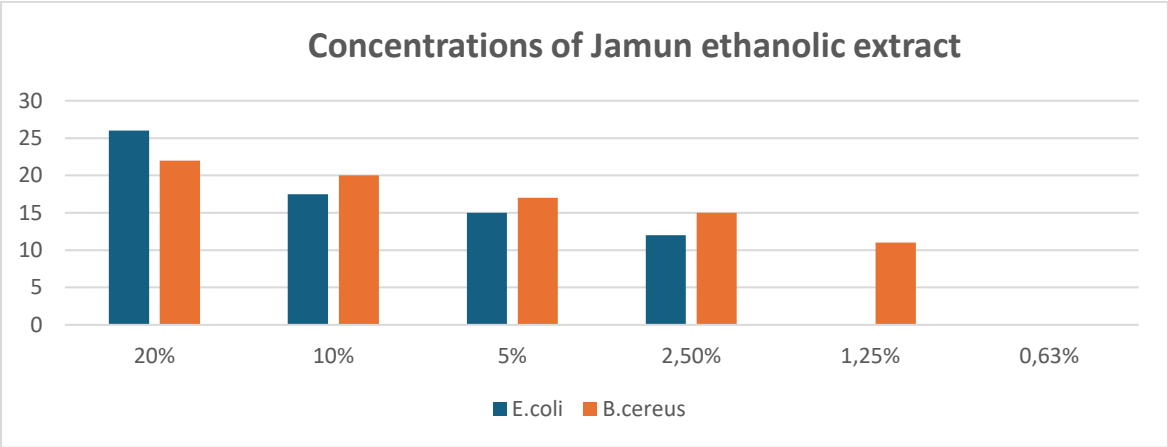


Figure 6. Antibacterial activity of Synergistic effect of Ethanolic and Distilled Water extracts of Jamun and Cinnamon (a) *E. coli* and (b) *B.cereus*

Figure 6. Antibacterial activity of Synergistic effect of Ethanolic and Distilled Water extracts of Jamun and Cinnamon (a) E. coliand (b) B.cereus



Graph 1. Minimum Inhibitory Concentration of Jamun leaves plant extract

Phytochemical Analysis of Jamun plant extract

The phytochemical screening of the Jamun leaves ethanolic extract showed the presence of alkaloids, saponins, phytosterols, triterpenes, phenols, and flavonoidsand absence of carbohydrates, proteins and amino acids (Table V).

Table V.Phytochemical Analysis of Jamun leaves ethanolic extract

S. No.	Compounds	Test	Result
1	Alkaloids	Hager's Test	+
2	Carbohydrates	Benedict's Test	-
3	Saponins	Froth Test	+
4	Phytosterol	Liebermann Burchard	+
5	Triterpenes	Salkowski's Test	+
6	Phenols	Ferric Chloride Test	+
7	Flavonoids	Lead Acetate Test	+
8	Proteins	Xanthoproteic Test	-
9	Amino Acids	Ninhydrin	-

+: Presence; -: Absence

Discussion

The present research work was attempted to evaluate the role of medicinal plants in controlling micro bacterial contamination and to extend the shelf life of the stored food materials. Also, the present study demonstrated that extracts of Jamun and Cinnamon showed prominent antibacterial activity against Escherichia coli and Bacillus cereus, which

was in accordance with various studies conducted earlier. The inhibition zones for Jamun extract were 23 mm against E. coli and 17 mm againstB.cereus; for Cinnamon, the inhibition zones were 11 mm against E. coli and 22 mm againstB.cereus. These results are in concordance with the findings of Trivedi et al.(2015) showing potent antibacterial activity of Jamun and Cinnamon against such pathogens.

The synergistic effect of combining Jamun and Cinnamon extracts revealed an enhanced Inhibition zone of 22 mm against E. coli, which suggests a potential for improved antibacterial efficacy when these extracts are used together. This finding supports the potential for synergistic interactions between plant extracts, as noted in similar studies. For instance, research by Silva et al. (2010) highlighted the benefits of synergistic combinations of natural extracts to overcome microbial resistance. However, the combination did not show significant inhibition against B.cereus in either ethanol or water extracts, which indicates that the synergistic effect may be more pronounced for specific bacterial targets.

In contrast, the chemical preservatives, namely acetic acid and benzoic acid, did not show any antibacterial activity against the test bacteria, probably due to possible resistance to these chemicals. This is in agreement with the work previously done by Fiedler et al. in 2019, thereby raising concerns as put across by the World Health Organization on the increased antibiotic resistance. The study also highlighted increasing resistance of E. coli to commonly used antibiotics, thus confirming the conclusion of Tadesse et al. (2021) and Arbab et al. (2022) that stated high resistance of E. coli to multiple antibacterial drugs.

In the present study, the MIC values for Jamun were less compared to that of Patel et al. (2012), which indicated more potency of the extract in our experimental setup.The results of phytochemical screening for Jamun presented key bioactive compounds, such as alkaloids, saponins, flavonoids, phenols, and triterpenes, which were similar to that obtained by Ramos et al (2022). These discrepancies might result from the extraction methodology as well as variation in the plant material.The efficacy of Jamun and Cinnamon, especially synergistic potential, justifies their application as natural preservatives with an alternate choice to synthetic additives. The present study therefore strengthens the concept of exploring plant-based solution for improving the safety and quality of food. Further research should focus on in-vivo applications and detailed mechanisms of these extracts in order to make full use of the potential of these extracts for antibacterial applications.

Conclusion

In the present study, the agar well diffusion method was used to assess the antibacterial activity of 9 selected plant extracts, namely Jamun, Cinnamon, Black pepper, Timur, Marigold, Bel Patra, Saunth, Sauf and Mulethi. Out of the 9 plant extracts, the maximum

zone of inhibition was exhibited by the ethanolic extract of Jamun leaves, followed by Cinnamon, with an inhibition zone of 23 mm against E. coliand 17 mm against B.cereus, and 22 mm against B.cereusand 11 mm against E. coli, respectively. Chemical preservatives like acetic acid and benzoic acid did not show any remarkable antibacterial activity against the tested bacteria, thereby showing possible resistance or ineffectiveness. Gentamycin showed maximum inhibitory activity against both test bacteria followed by chloramphenicol and erythromycin andE. coli showed quite high resistance to many antibiotics and hence, there is a need for other alternative options. Minimum Inhibitory Concentration (MIC) obtained for Jamun extract against B.cereus was 1.25% and against E. coli, it was 2.5%. The synergistic activity of ethanolic extracts of Jamun and Cinnamon showed a combined inhibition zone of 22 mm against E. coli, thus, showing potential effectiveness when used together. The activity of Jamun ethanolic extract may be attributed to the presence of alkaloids, saponins, phytosterols, triterpenes, phenols, and flavonoids. This study therefore underlines the potential of plant-based extracts as effective alternatives to chemical preservatives and antibiotics in fighting bacterial food spoilage and infections and hence highlighting the need for further research into their mechanisms of action.

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