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Antibacterial Screening of Medicinal Plant Extracts against *Staphylococcus aureus* and *Escherichia coli*

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Authors' contributions

This work was carried out in collaboration among all authors. Author CAB performed material preparation, research work, data analysis, interpretation, and manuscript writing. Authors EMM and PC participated in the suggestions and critical reviews. All authors read and approved the final manuscript.

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ABSTRACT

Herbal remedies are crucial for treating bacterial infections due to antibiotic resistance, with numerous studies demonstrating their antibacterial properties against various harmful species. This study investigates the antibacterial properties of an aqueous extract from 40 medicinal plants *in vitro*, assessing their effectiveness against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The well diffusion method was employed to assess the antibacterial activity. Six plants exhibit antibacterial properties against the two bacterial strains among these extracts. Guava (*Psidium guajava*) and Clove (*Syzygium aromaticum*)cold water extracts were the most effective plant extracts against *Escherichia coli*, with inhibition zones of 6.2

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mm and 5.2 mm, respectively. Coriander (*Coriandrum sativum*) leaves and Sana makki (*Cassia angustifolia*) leaves cold water extracts were the most effective plant extracts against *Staphylococcus aureus* with an inhibitory effect with inhibition zones of 6.9 mm and 5.9 mm respectively. The plant extracts differ in antibacterial activities against *S. aureus* and *E. coli*. However, Extracts of *Senna alata*, *Tamarindus indica*, *Psidium guajava*, *Syzygium aromaticum*, and *Cassia angustifolia* showed inhibitory activity against both of the microorganisms tested. These potentially useful plant extracts can be utilized as natural alternatives to preventive measures for food poisoning and preservation, thereby avoiding the health risks associated with chemically antibacterial agents. These antibacterial properties encourage future research into new chemical compositions that can help treat or cure some diseases.

Keywords: Antibacterial activity; Staphylococcus aureus; Escherichia coli; Psidium guajava; Coriandrum sativum; well diffusion method.

1. INTRODUCTION

Men have used plants as a source of food, housing, clothing, medicine, cosmetics, and as a means of seeking solace from the hardships of life in an ongoing effort to better their quality of life. On Earth, there are thought to be between 250,000 and 500,000 plant species, some of which have been extensively used in medicine. Research on medicinal plants has received a lot of interest recently. Different elements of plant chemicals' antibacterial, antifungal, antiviral, and antiprotozoal activities have been studied. Traditional herbal medicine practitioners have documented many native plants' therapeutic effectiveness for various ailments. Because plants contain a variety of components necessary for life, scientists have been compelled to study them to identify any potential medical benefits [1]. In many medical systems, plants have a ubiquitous role in treating diseases. Numerous plant species are used by various indigenous medical systems, including Ayurveda, Sidda, Homoeopathy, and Naturopathy, to treat multiple illnesses [2]. Antimicrobial compounds can be found in abundance in medicinal plants. Different nations employ plants as medicine, which are the source of several powerful drugs [3].

Mankind has utilized many types of plants for many years to treat a wide range of diseases because they are known for their propensity to produce a multitude of secondary metabolites. The therapeutic effects of herbs are believed to be caused by secondary metabolites having in situ functions such as growth regulation, inter- and intra-specific interactions, and defense against pathogens and predators. Many of these natural compounds are used as chemotherapeutic agents or as components for the production of contemporary pharmaceuticals because it has been demonstrated that they exhibit fascinating biological and pharmacological activity [4, 5]. Herbs are recognized as a major natural source of medicines worldwide because they are secure, less poisonous, affordable, and reliable [6]. In addition to being costly and ineffective for treating disease, synthetic medications also have a high risk of adulteration and adverse effects. Therefore, it is necessary to look for innovative, safe infection-fighting techniques to manage microbial infection. For new and re-emerging infectious diseases, there is an ongoing and urgent need to find new antimicrobial agents with different chemical structures and unique modes of action [7]. As a result, researchers are increasingly focusing on traditional medicine to find novel ideas for creating more effective medications to treat microbial illnesses.

Antibacterial resistance has become a significant issue in modern medicine. Alternative therapies, such as using plant extracts with therapeutic characteristics, have become increasingly popular. For example, cinnamon extract was discovered to be the most effective spice against every tested strain, while cumin, ginger, and clove, in that order, showed the poorest antibacterial activity [8]. Other researchers confirmed that clove oil was active against all pathogenic bacteria tested but discovered that aqueous clove extract was ineffective against *Vibrio cholera, S. typhi*, and *Klebsiella pneumonia* [9.10]. Moreover, the methanolic clove extract was reported to be potentially effective against *S. aureus*, *P. aeruginosa* and *E. coli* with Minimum inhibitory concentration (MIC) ranging from 0.1 to 2.31 mg/ml [11]. In this project, we will be exploring the potential of medicinal plant extracts as antibacterial agents against gram-negative *E. Coli*, and gram-positive *Staphylococcus,* the most common bacterial

pathogens. We aim to investigate different plant extracts' effectiveness and identify the most potent extracts for further development as natural antibacterial agents. Through this research, we hope to contribute to developing alternative treatments for bacterial infections and address the growing issue of antibiotic resistance.

2. MATERIALS AND METHODS

2.1 Materials Required

2.1.1 Chemicals

1. Mueller- Hinton agar media (hi media Laboratories, Pvt. Ltd.)
2. Luria-Bertani broth media

2.2 Collection of Plant Materials

The respective plant parts of 40 plant species were collected from different locations in the Malappuram district of Kerala, while some were purchased from grocery stores. The sample collection was done in January (2023), and the collected plant parts were washed and dried in the shade. The dried plant material of each plant species was ground into a fine powder using an electronic grinder and sieved with a 2 mm diameter mesh. The plant powders were stored in separate bottles. Two grams of each plant powder were weighed using an electric balance and taken into separate conical flasks, and 20 ml of distilled water was added. The samples with solvent were kept for 24 hours. The contents of conical flasks were then filtered into 15ml centrifuge tubes using Whatmann No 1 filter paper. Store the extracts at 4°C until use.

2.3 Bacterial Strains

2.3.1 Microorganisms used

Gram positive - *Staphylococcus aureus*
Gram-negative - *Escherichia coli*

2.4 Sterilization

The glass wares, inoculation loop, petri plates, watch glasses, test tubes, and conical flasks were washed well in distilled water and autoclaved. The aseptic condition was maintained in all steps of inoculation and incubation.

2.5 Medium Preparation

The Mueller Hinton agar medium was prepared by suspending 19g of MH agar medium in 500 ml of distilled water and boiling it to dissolve completely. The medium was then sterilized by autoclaving at 15lbs pressure (121°c) for 15 minutes and mixed well before pouring. The medium was cooled, and 20ml were distributed into each sterile petri dish.

2.6 Inoculums Preparation

Bacterial inoculums were prepared in a 3ml nutrient broth medium and incubated at 37°c for 24 hours.

2.7 Inoculation

The stock cultures of microorganisms used in the study were maintained by agar slants at 4°c. Inoculums were prepared by suspending a loop full of bacterial cultures into 3ml of nutrient broth and were incubated at 37°c for 2-4 hrs. The bacterial suspension was poured into Petri plates containing 20 Muller-Hinton agar medium. The bacterial suspensions were spread to get a uniform lawn culture.

2.8 Antibacterial Assay Using Well Diffusion Methods

The antibacterial activity of water extracts was determined by the well diffusion method [12]. Sterile liquid nutrient agar media was transferred into sterile petri plates, and after solidification, the bacteria were swabbed under aseptic conditions. Wells of 4 mm diameter were cut into Solidified agar media. Four wells were made on each plate, and each was added with 40µl plant extracts using a micropipette, one for solvent (water) control and one for positive control (Gentamicin). The plates were incubated at 37°c for 24hrs, and the zone developments were closely monitored. After incubation, the antibacterial activities of the plant extracts against the microbes were assessed by measuring the diameter of the incubation zone formed. The diameter of the inhibition zone thus obtained was measured.

2.9 Determination of Minimum Inhibitory Concentrations (MICs) of the Effective Plant Extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits microbial growth after 24 h. of incubation. The most effective plant

extracts that exhibit strong antibacterial activity are manipulated to determine their MIC using a good diffusion method and evaluate their efficiency in controlling bacterial strains. Different concentrations of the effective plant extract (0.25, 0.5, 1, 2, and 4 mg/ml) were prepared separately by dissolving 4 mg in 10 ml of distilled water and loading their requisite amount over sterilized filter paper discs (8 mm in diameter) for sterilization. Mueller-Hilton agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. 40µl plant extracts were loaded to each well with different concentrations of the effective plant extract and then incubated at 35°C for 24 h. The inhibition zones were measured using a scale and recorded against the concentrations of the effective plant extracts.

3. RESULTS

This study reports the antimicrobial activity of 40 plant extracts from 40 selected medicinal plants against two strains of bacteria, including *Escherichia coli* and *Staphylococcus*. The existence or absence of an inhibition zone and the diameter of the inhibition zone surrounding the wells were used to quantitatively evaluate the extracts' antibacterial activity. Plant extracts' antibacterial activity findings are displayed in Table 1. The most pronounced activity with an inhibition zone of 10 mm was shown by coriander leaf (*Coriandrum sativum*) and sunnamakki (*Cassia angustifolia*) against *Staphylococcus*. At the same time, coriander leaf (*Coriandrum sativum*) forms only a 6 mm inhibition zone against *E. Coli.*

Similarly, the guava leaf (*Psidium guajava*) showed an inhibition zone of 9mm against *Staphylococcus aureus*. The clove (*Syzygium aromaticum*) and anathakara (*Senna alata*) showed the same range of inhibition zone against both *E. Coli* and *Staphylococcus aureus*. The plant extracts of tamarind leaves (*Tamarindus indica*) and guava leaves (*Psidium guajava*) showed an inhibition zone of 7mm against *Staphylococcus*. Intern tamarind leaves (*Tamarindus indica*) showed an inhibition zone of 6 mm against *E.coli.* Least activity is shown by canary seeds (*Phalaris canariensis*) by forming an inhibition zone of 5 mm against *E. Coli*. (Table 1, Fig. 1 &2). This study has concluded that Coriander (*Coriandrum sativum*) leaves and sunnamakki (*Cassia angustifolia*) show the most anti-bacterial activity towards *Staphylococcus aureus* and the guava (*Psidium guajava*) leaf highest growth inhibition towards *Escherichia coli* among the tested 40 plant extracts. The tested plant extracts differ in their antibacterial activities against *S. aureus* and *E. coli.* However, Extracts of *Senna alata, Tamarindus indica, Psidium guajava, Syzygium aromaticum, Cassia angustifolia* showed inhibitory activity against both microorganisms tested.

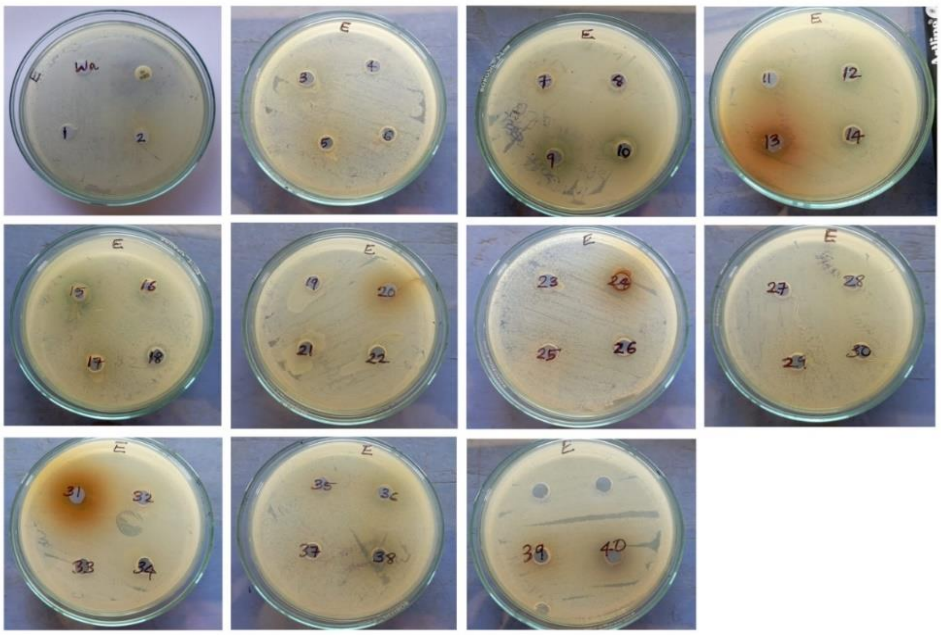

Fig. 1. Growth inhibition of *Escherichia coli* strains caused by plant extracts, Wa-water (solvent) control, Gen- Gentamicin positive control & Plant extracts (1-40)

Table 1. Inhibition zone (mm)of 40 plant extracts on *Escherichia coli* and *Staphylococcus aureus*

Sl. No	Plant extracts			Effective zone of inhibition* (in mm diameter)	
	Common name(Kerala)	Scientific name	Used plant part	*E. coli*	*S. aureus*
1	Garlic	*Allium sativum*	Bulb	-	-
2	Poovamkurunnila/little iron weed	*Cyanthillium cinereum*	Leaves	-	-
3	Coriander leaf	*Coriandrum sativum*	Leaves	-	10
4	Jathika	*Myristica fragrans*	Seeds	-	-
5	Gooseberry	*Emblica Officinalis*	Fruits	-	-
6	Brahmi	*Bacopa monnieri*	Leaves	-	-
7	Samgupushpam	*Echinacea purpurea*	Leaves	-	-
8	Ayamodhakam	*Trachyspermum ammi*	Leaves	-	-
9	Papaya	*Carica papaya*	Leaves	-	-
10	Passiflora edulis	*Passion fruit*	Leaves	-	-
11	Idinjil	*Commiphora caudate*	Leaves	-	-
12	Ginger	*Zingiber officinale*	Rhizome	-	-
13	Anathagara	*Senna alata*	Leaves	7	7
14	Aashali (Garden cress)	*Lepidium sativum*	Seeds	-	-
15	Drumstick	*Moringa oleifera*	Leaves	-	-
16	Kaidhola	*pandanus amaryllifolius*	Leaves	-	-
17	Tulasi	*Ocimum tenuiflorum L.*	Leaves	-	-
18	Tamarind	*Tamarindus indica*	Leaves	6	7
19	Mukkuty	*Biophytum sensitive*	Leaves	-	-
20	Karinechi	*Vitex negundo*	Leaves	-	-
21	Black cumin	*Nigella sativa*	Seeds	-	-
22	Aryaveppu	*Azadirachta indica*	Leaves	-	-
23	Jeerakam	*Foeniculum vulgare*	Seeds	-	-
24	Guava	*Psidium guajava*	Leaves	9	7
25	Keezhar nelli	*Phyllanthus niruri*	Whole plant	-	-
26	Adalodakam	*Justicia adathoda*	Leaves	-	-
27	Black pepper	*Embelia ribes*	Seeds	-	-
28	Orange	*Citrus reticulate*	Pericarp	-	-
29	Cardamom	*Elettaria cardamomum*	Pod	-	-
30	Turmeric	*Curcuma longa*	Rhizome	-	-
31	Clove	*Syzygium aromaticum*	Flower buds	8	8
32	Pomelo	*Citrus maxima*	Peel	-	-
33	Bitter gourd	*Momordica charantia*	Fruit	-	-
34	Puncture vine(Njerinjil)	*Tribulus Terrestris*	Seeds	-	-
35	Canary Seeds(kattujeerakam)	*Phalaris canariensis*	Seeds	5	-
36	Bay leaf	*Laurus nobilis*	Leaves	-	-
37	Cinnamon	*Cinnamomum verum*	Bark	-	-
38	Soursop	*Annona muricata*	Leaves	-	-
39	Thechi	*Ixora coccinea*	flowers and leaf	-	-
40	Sana makki	*Cassia angustifolia*	Leaves	6	10

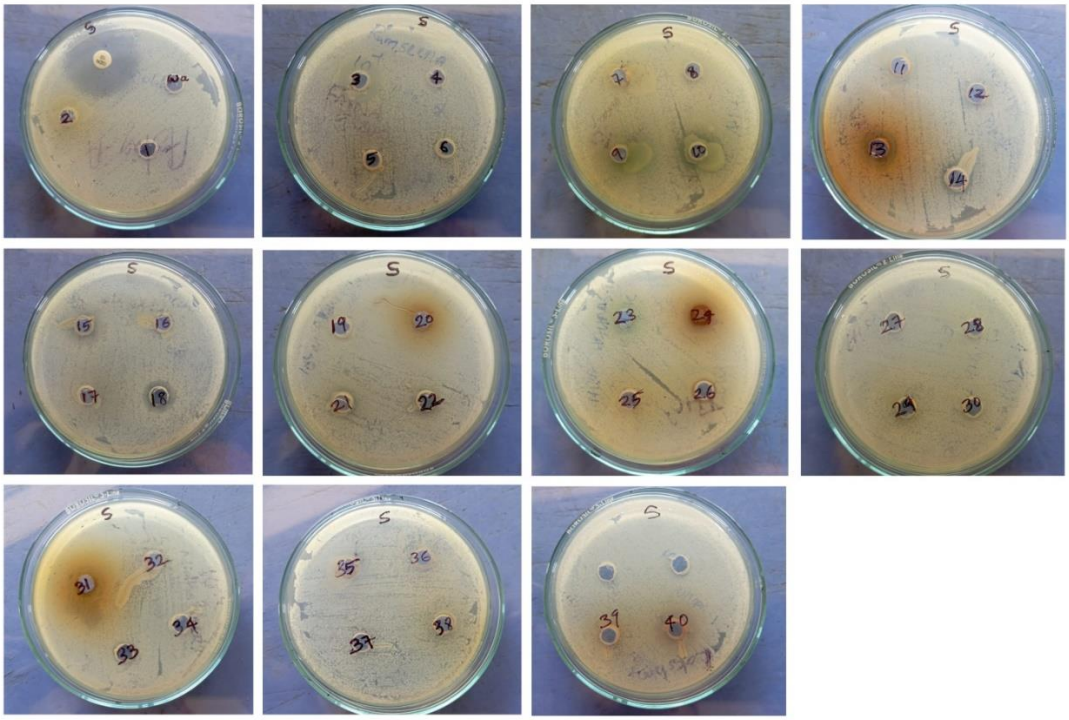

Fig. 2. Growth inhibition of *Staphylococcus aureus* strains caused by plant extracts, Wa-water (solvent) control, Gen- Gentamicin positive control & Plant extracts (1-40)

Table 2. MICs of the most effective plant extracts against *E. coli*

Plant extracts	Conc. (mg/ml)	Inhibition zones (mm)
Guava (*P. guajava)*	0.25	0.0±0.0
	0.5	6.2±0.71
	1	9.3±0.52
	2	14.5±0.48
	4	23.1±0.78
Clove (*S. aromaticum)*	0.25	0±0.0
	0.5	5.2±0.31
	1	8.7±0.64
	2	13.8±0.56
	4	21.4±0.83

3.1 Minimum Inhibitory Concentrations (MICs) of the Effective Plants Extract against *Escherichia coli*

The MIC of the most effective two plant extracts (*P. guajava* and *S. aromaticum*) was employed by the well diffusion method to evaluate their bactericidal properties. The inhibitory effect of *P. guajava* extract started at 0.5 mg/ml with inhibition zones of 6.2 mm against *E. coli*. The extract of *S. aromaticum* suppressed bacterial growth of these strains at a concentration of 0.5 mg/ml with inhibition zones of 5.2 mm.

3.2 Minimum Inhibitory Concentrations (MICs) of the Effective Plants Extract against *Staphylococcus aureus*

The MIC of the most effective two plant extracts *C. sativum* and *C. angustifolia* was employed by the well diffusion method to evaluate their bactericidal properties. The inhibitory effect of *C. sativum* extract started at 0.5 mg/ml with inhibition zones of 6.9 mm against *S. aureus*. The extract of *C. angustifolia* suppressed bacterial growth of these strains at a concentration of 0.5 mg/ml with inhibition zones of 5.9 mm, respectively.

Table 3. MICs of the most effective plant extracts against *S. aureus*

Plant extracts	Conc. mg/ml	Inhibition zones (mm)
Coriander leaf (*C. sativum)*	0.25	0.0±0.0
	0.5	6.9±0.62
	1	10.1±0.52
	2	19.5±0.29
	4	26.1±0.34
Sana makki (*C. angustifolia)*	0.25	0.0±0.0
	0.5	5.9±0.19
	1	10±0.73
	2	17.8±0.56
	4	25.4±0.83

4. DISCUSSION

Plant extracts are a complex mixture of several chemical components with varying intensities. The biological effects of the extracts vary since these substances have undergone extensive modifications. Plant extracts are gaining much interest as an appropriate antibiotic alternative for prospective therapeutic targets, health and beauty goods, and the food sector due to the antimicrobial capabilities of various herbal extracts and other biological effects [13]. The present study was designed to obtain preliminary information on the antimicrobial effect of extracts from 40 selected medicinal plants against two strains of bacteria, including *Escherichia coli* and *Staphylococcus aureus*. Extracts of *Senna alata, Tamarindus indica, Psidium guajava, Syzygium aromaticum, Cassia angustifolia* showed inhibitory activity against both of the microorganisms tested. The well diffusion method was preferred for this study. The most pronounced activity was shown by coriander leaf and sannamakki against *Staphylococcus aureus*. Similarly, the guava leaf showed an inhibition against *Staphylococcus aureus.* The clove (*Syzygium aromaticum*) and anathakara (*senna alata*) showed the same range of inhibition zone against both *E. coli* and *Staphylococcus aureus*. Research on the antimicrobial activities of the plant's essential oils has revealed that coriander is a medicinal herb with potent antibacterial activity [14]. These findings agree with those of [15 - 17].

A significant difference in the MIC of extracts was found in numerous experiments; this could be because of the ingredients, bacterial strains, and extraction techniques used. Additionally, variations in chemical contents and the volatile nature of ingredients may contribute to variations in MIC of various plant extracts. As can be seen from Table 2, the extract of *S. aromaticum* suppressed bacterial growth of *E. coli* strains at a concentration of 0.5 mg/ml with inhibition zones of 5.2 mm. Mostafa *et al.* reported that the growth of *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa* was shown to be inhibited by *S. aromaticum* extract at concentrations of (10 mg/ml), with inhibition zones measuring 14.6, 15.8, 11.9, and 13.4 mm, respectively [18]. These findings are consistent with those of Mahfuzul_Hoque *et al.* indicated that the maximum inhibition of ethanol extracts of guava for *S. aureus* JCM 2151 (0.1 mg/mL) *L. monocytogenes* JCM 7676 (0.1 mg/mL), *V. parahaemolyticus* IFO 12711 (0.1 mg/mL), and *S. aureus* JCM 2179 (0.1 mg/mL) [19]. Pandey and Singh (2011). Comparing these findings to those obtained by Dua *et al.* (2013), it was discovered that cumin was unsuccessful in suppressing the other bacterial strains. MICs of cumin ranging from 6.25 to 12.5 mg/ml.

On the other hand, a more significant concentration of cumin extract, up to 60 mg/ml, might be needed to be effective against bacteria that cause food spoilage. The results of this study matched those previously reported by Sheikh *et al.* [20]. Numerous research studies have looked at plant extracts' effectiveness and antimicrobial properties in preventing the growth of bacteria that cause food poisoning and spoiling. Some researchers have proposed that terpenoid, alkaloid, and phenolic compounds found in plant extracts, which have antimicrobial properties, interact with the enzymes and proteins that make up the microbial cell membrane, disrupting them to release protons that cause cell death or may prevent the enzymes needed for the biosynthesis of amino acids [21,22]. The hydrophobicity properties of these plant extracts, which allow them to react with proteins of the microbial cell membrane and

mitochondria to upset their structures and alter their permeability, have an inhibitory effect [23, 24].

According to Matasyoh's study, the essential oils from coriander leaves had antibacterial and antifungal properties against *C. albicans*, gram-positive and gram-negative bacteria. Additionally, its main constituents were aldehydes and alcohols, which comprised 55.5% and 36.3% of the oil, respectively, and linalool, which comprised 0.32% of the oil [25]. This study revealed that the linalool content in coriander leaves' essential oils was lower than that of coriander fruits' essential oils, but aldehydes and alcohols were higher than in fruits' essential oils. It also revealed that other essential oil constituents can act against microbes. The most potent essential oil component in cloves for antibacterial activity is eugenol. The way in which antimicrobial action involves targeting the phospholipid found in the cell membrane. Essential oils have bacteriostatic and bactericidal properties that inhibit microbes [26]. *C. angustifolia* is antibacterial against *Shigella shinga*, *Klebsiella pneumonia*, and *E. coli*. The flavonoids quercimeritrin, scutellarein, and rutin found in methanol, ethanol, and ethyl extracts give them antibacterial properties [27]. Other recent reports on antimicrobial activity of *Senna alata* leaf extract to *Escherichia coli*, *Streptococci pyogenes*, and *Proteus mirabilis* also supported this study.

According to the results of the above discussed studies, the observed antimicrobial activity of the plant extracts of *Senna alata, Tamarindus indica, Psidium guajava, Syzygium aromaticum, Cassia angustifolia* may be due to the presence of potent phytoconstituents in the extracts. These plant extracts have shown promise as natural preservatives and can be utilized to prevent food poisoning and preserve food without causing any health risks.

5. CONCLUSION

The present study revealed that significant anti-bacterial activity was observed in six plant extracts among the 40 different plant extracts studied against *S. aureus* and *E. coli*. This study has concluded that Coriander (*Coriandrum sativum*) leaves and sunnamakki (*Cassia angustifolia*) show the most anti-bacterial activity towards *Staphylococcus aureus* and the guava (*Psidium guajava*) leaf highest growth inhibition towards *Escherichia coli* among the tested 40 plant extracts. We used the cold water extraction technique in this study. So, this extract preparation is accessible to the general public for daily use. Plant extracts that have shown antimicrobial activity can be utilized to prevent food poisoning and preserve food without causing any health risks. It can also be applied to specific food or products with a prolonged shelf life and avoid the health risks of using chemical antimicrobial agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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