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Evaluation of antimicrobial activity of Crude Ethanolic Extracts of Selected Spices

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ABSTRACT

Infectious diseases and food poisoning caused by microbial pathogens continue to pose significant threats to global public health. In this context, the antimicrobial properties of certain spices have garnered attention due to their historical use not only for culinary purposes but also for their medicinal value. This study aimed to assess the antimicrobial activity of crude ethanol extracts obtained from a selection of spices, including clove, cinnamon, cardamom, fenugreek, cumin, and ajowan against several pathogenic bacteria, namely *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*.

Results indicated that among the six tested spices, clove exhibited the highest antimicrobial efficacy, with notable inhibition zones of 23mm against *E. coli*, 20mm *against Klebsiella pneumoniae*, 32mm against *Staphylococcus aureus*, and 16mm against *Pseudomonas aeruginosa*. Cumin demonstrated considerable antimicrobial activity with inhibition zones of 22mm, 14mm, 29mm, and 20mm against *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, respectively. Ajowan, cardamom, and cinnamon also displayed varying degrees of antimicrobial efficacy against the tested bacterial strains.

On the other hand, fenugreek showed weak antimicrobial activity, as it failed to inhibit the growth of any of the four bacterial isolates. The findings suggest that spices such as clove, cumin, and cinnamon possess promising potential as natural remedies against pathogenic bacteria, providing a safer alternative to traditional antibiotics. Additionally, these spices could serve as natural preservatives in various food and healthcare applications.

Keywords: Spices, Ethanol extract, Antimicrobial activity, Antibiotics.

INTRODUCTION

Spices have been treasured for millennia across diverse cultures for their ability to enhance the flavor and aroma of food. Besides their culinary appeal, ancient societies recognized the medicinal and food preservation properties of spices (Ali B et al., 2015). In recent years, modern science has started to unveil the anti-microbial effects of various spices, adding to their appeal as potential remedies for combating bacterial pathogens (Ceylan and Fung, 2004).

The widespread use of antibiotics has led to a concerning rise in bacterial resistance. As a result, traditional medicine has garnered renewed interest as a viable alternative for treating various diseases while avoiding the side effects associated with conventional treatments (Saeed and Tariq, 2007). At present, it is estimated that about 80% of the world's population relies on botanical preparations as medicines to meet their health needs (WHO 2002). Spices are eventually considered safe and proved to be effective against certain pathogenic bacteria. It is only in recent years that modern science has started paying attention to the properties of spices (Chaudhry and Tariq 2006). They are also extensively used mainly in many Asian, African and other countries. In recent years, because of their beneficial effects, the use of spices has been increasing in developed countries also (Indu *et al.* 2006).

In the realm of food safety, microbial pathogens pose a significant threat to both food spoilage and foodborne illnesses. The rise of multidrug-resistant bacteria like *Pseudomonas aeruginosa* and *Escherichia coli* has escalated morbidity and mortality rates (Miladi et al., 2016). To address this growing concern, there is a shift towards using natural products, such as spices and their extracts, as substitutes for synthetic chemical preservatives in the food industry. These natural alternatives offer better tolerability in the human body and exhibit superior properties for food preservation (Silva et al., 2017).

In light of the alarming impact of infectious diseases, particularly due to antibiotic resistance, it becomes crucial to explore effective and safe alternatives for treatment. Spices and their extracts present a promising avenue, displaying anti-microbial activity attributed to various bioactive compounds, including alkaloids, flavonoids, tannins, and phenolic compounds (Hoque et al., 2008). Phytochemicals, or secondary metabolites, found in spices further enhance their antimicrobial properties, serving as protectants against harmful organisms and responding to environmental changes (Avato et al., 2002).

This research aims to investigate the anti-microbial activity of selected spices—Clove, Cinnamon, Cardamom, Ajowan, Fenugreek, and Cumin—against pathogenic bacteria like Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae. By exploring the potential of these spices in combating bacterial pathogens, this study contributes to the broader understanding of their applications in enhancing food safety and possibly addressing antibiotic resistance challenges.

Methodology

3.1 Study Duration and Laboratory Settings

This study was conducted over a period of three months, from Feb - Apr 2022. The extraction phase took place in the chemistry laboratory of Birendra Multiple Campus, while the anti-microbial activity testing was carried out in the Microbiology laboratory of Balkumari College, Chitwan.

3.2 Sample Selection and Collection

Six commonly used spices (clove, cumin, cardamom, cinnamon, ajowan, fenugreek) were randomly sampled from Bharatpur's market, Chitwan, based on traditional medicinal use.

3.3 Sample Processing

The collected samples were thoroughly cleaned and subjected to shade drying on blotting paper in a dark room until completely dry. After drying, the sample was packed in waterproof bags and stored at room temperature away from direct sunlight. The dried samples were ground to obtain fine powder using a grinder.

3.4 Soxhlet Extraction with Dehydrated Ethanol

The Soxhlet extraction process was performed in the Department of Chemistry, Birendra Multiple Campus, Chitwan. Dried plant powder was weighed and loaded into a clean and dried thimble of the Soxhlet extractor. A round-bottom flask, previously weighed and labeled, was fitted with the thimble. Ethanol (150 ml) was slowly poured into the soxhlet extractor, and the setup was fitted with a condenser. The flask was heated with a heating mantle, allowing the solvent vapors to reach the spices powder and dissolve the soluble compounds. The condensed solvent dropped back into the flask, resulting in a colored solvent solution. The process ran for 8-15 hours or until the desired extract was obtained.

3.5 Removal of Solvent

After extraction, the solvent was removed from the round-bottom flask using a rotatory vacuum evaporator under negative pressure. The flask was constantly heated in a rotating condition using a water bath below 55°C. The solvent was completely evaporated, collected in a separate round-bottom flask, and stored in a sterile bottle. The extract's yield was determined by weighing the flask before and after extraction.

3.6 Preparation of Working Solution

One gram of crude ethanol extract from each spice was aseptically transferred into a sterile 20 ml screw cap test tube. 9 ml of sterile distilled water was added to each tube, and the mixture was vortexed to obtain a homogenous solution of 100 mg/ml working suspension, which was stored in a refrigerator at 2-8°C.

3.7 Collection of Bacterial Cultures

Four different human pathogenic bacteria were selected for the study, namely *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumonia* ATCC 200603, and *Pseudomonas aeruginosa* ATCC 27853. The bacterial cultures were obtained from the Department of Microbiology, Balkumari College, Chitwan. After obtaining the cultures, they were streaked on nutrient agar plates and incubated at 37°C for 24 hours. Gram staining and biochemical tests were performed to confirm the purity of the isolates.

3.8 Preparation of Standard Culture Inoculums

Standard culture inoculums were prepared by transferring three to five colonies of the selected organism into a tube containing sterile nutrient broth. The turbidity of the broth was adjusted to the turbidity of the broth was then adjusted to match the McFarland standard.

3.9 Preparation of Media

The media used in the study were prepared according to the manufacturer's recommendations.

3.10 Qualitative Screening and Determination of Anti-Bacterial Activity

The anti-bacterial activity of the crude spice extracts was determined using the agar well diffusion method (Dingle et al., 1953). Sterile Muller-Hinton Agar (MHA) plates were prepared with a thickness of approximately 4 mm. The plates were inoculated with the prepared inoculum and wells were made using a sterile cork borer. Working suspensions of the spice extracts were added to the wells, and the plates were incubated at 37°C for 18-24 hours. After incubation, the plates were observed for the presence of a zone of inhibition (ZOI) as a clear area without bacterial growth around the well. The ZOI was measured using a scale, and the mean value was recorded.

Result

4.1 Ethanolic extract of spices yield

The percentage yields of the ethanol extract of spices as obtained by Soxhlet extraction processes as shown in figure 1. Among the spices, clove exhibited the highest yield, with an impressive extraction efficiency of 40.7%. On the other hand, ajowan showed the lowest yield, with only 9.8% of the crude extract obtained during the extraction procedure. The amounts of crude extracts varied significantly across the different spices studied.

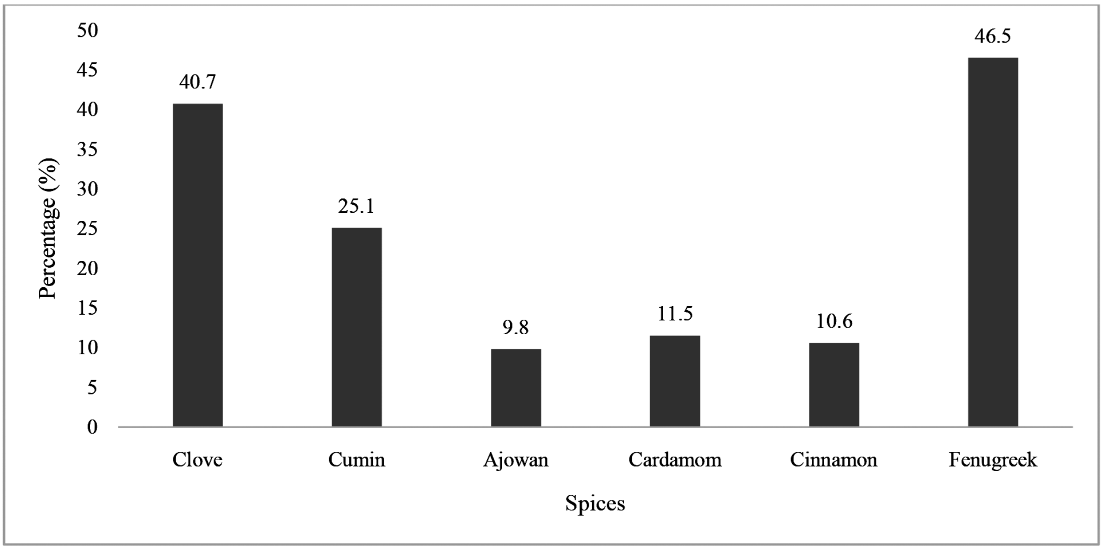

Figure 1: Percentage yields of ethanol crude extracts of spices.

4.2 Screening of spices for anti-bacterial activity

Table 1 displays the data concerning the antimicrobial efficacy of spice extracts. Among the six spices investigated (clove, cumin, cardamom, ajowan, and cinnamon), five exhibited significant effectiveness against all the tested pathogenic bacteria. These spices, namely clove, cumin, cardamom, ajowan, and cinnamon, demonstrated potent antimicrobial properties, indicating their potential as natural agents for combating pathogenic bacterial infections.

Table 1: Anti-bacterial properties of ethanolic extracts of different spices against tested bacteria.

S.N.	*Spices*	*E. coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
1	Clove	+	+	+	+
2	Cumin	+	+	+	+
3	Ajowan	+	+	+	+
4	Cardamom	+	+	+	+
5	Cinnamon	+	+	+	+
6	Fenugreek	-	-	-	-

Note: Positive (+) sign indicates the ethanolic extracts of the particular spices inhibited the growth of microorganisms and thus produce zone of inhibition. Absence of zone of inhibition was denoted as negative (-) sign.

4.3 Evaluation of antibacterial activity of spices

The anti-bacterial activities of medicinal plants were evaluated by measuring the zone of inhibition. The diameter of the zone of inhibition (ZOI) produced by spice extract and particular bacteria was measured for the estimate of potency of spice extracts. The mean diameter of zone inhibition of different spices which showed significant zone of inhibition (>8mm) during the qualitative screening process as indicated by the '+' sign is shown in figures below.

4.3.1 Anti-bacterial activity of cloves

The anti-bacterial activity of cloves was found to be remarkably robust, as it displayed effectiveness against all the tested bacterial isolates. This impressive result suggests that cloves may possess broad-spectrum antibacterial properties, meaning it has the ability to target and inhibit the growth of a wide range of pathogenic bacteria.

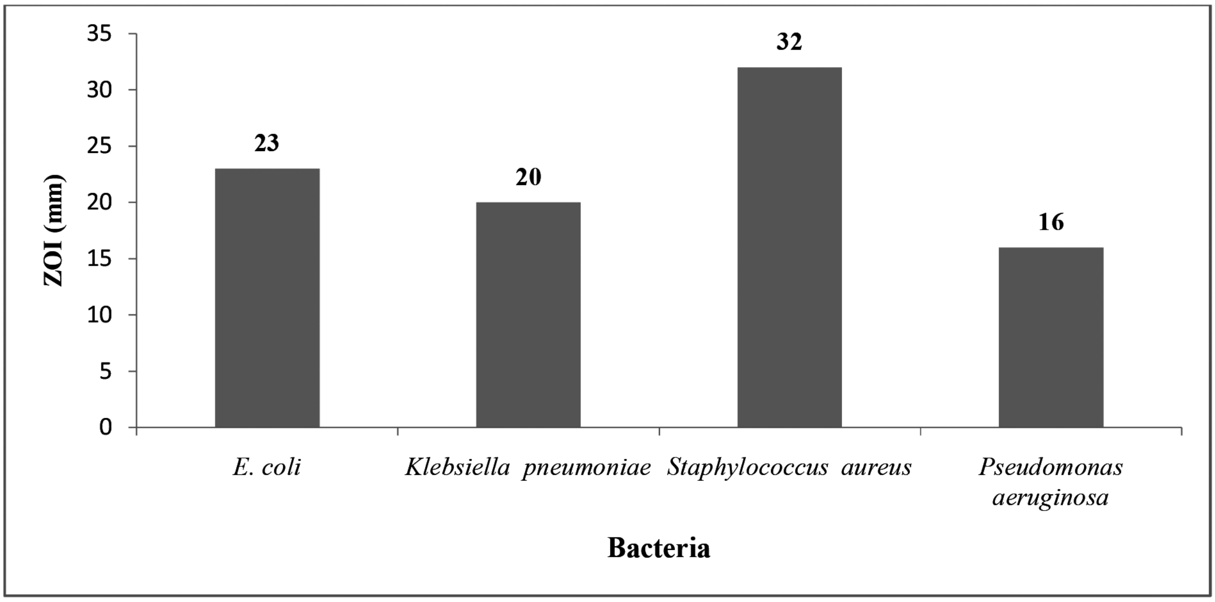

Figure 2: Zone of inhibition (ZOI) given by cloves against tested bacteria.

4.3.2 Anti-bacterial activity of cumin

Cumin demonstrates broad-spectrum antibacterial activity as it was found to inhibit the growth of four out of the tested bacterial organisms. This suggests that cumin's antibacterial properties are effective against a diverse range of pathogenic bacteria.

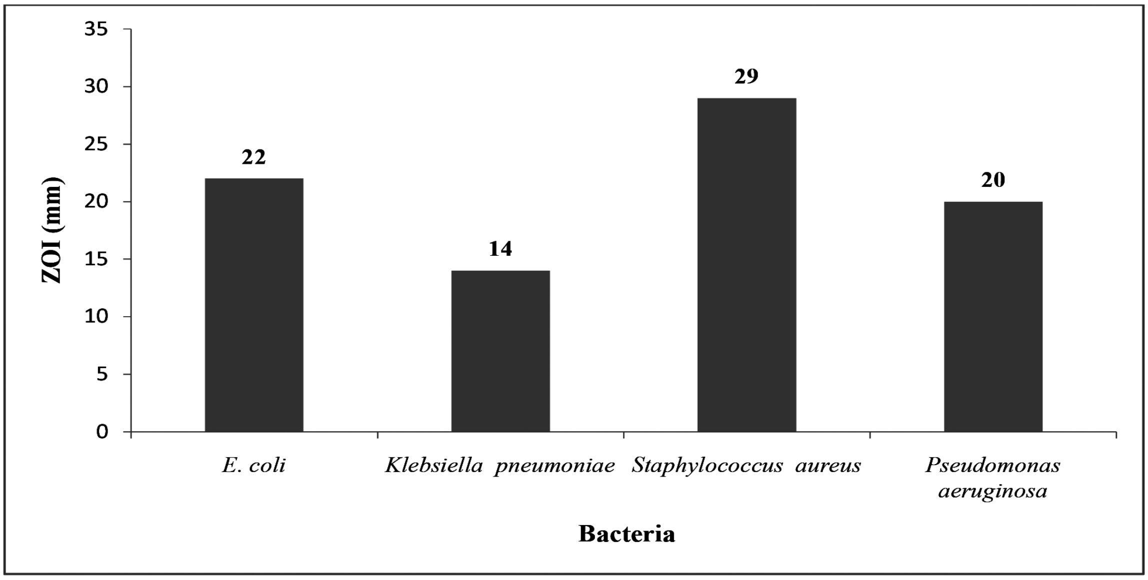

Figure 3: Zone of inhibition (ZOI) given by Cumin against tested bacteria.

4.3.3 Anti-bacterial activity of ajowan

The anti-bacterial activity of ajowan was observed to be limited, as it exhibited an inhibitory effect against only three out of the tested pathogenic bacterial isolates. The zone of inhibition (ZOI) for ajowan extract against *Pseudomonas* was relatively small, measuring only 7mm. This indicates that ajowan's inhibitory action was less pronounced against *Pseudomonas* compared to other bacterial isolates.

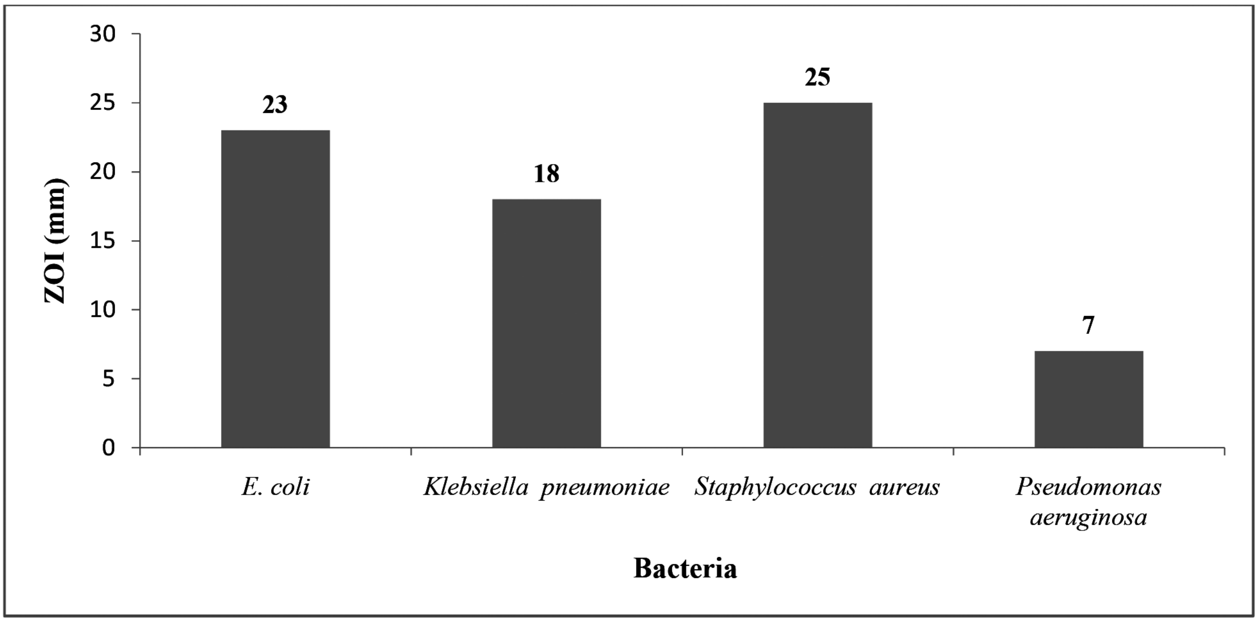

Figure 4: Zone of inhibition (ZOI) given by ajowan against tested bacteria.

4.3.4 Anti-bacterial activity of Cardamom

The anti-bacterial activity of cardamom was observed to be relatively limited, as it inhibited the growth of only three of the tested bacterial isolates. Among the bacteria tested, cardamom extract showed a zone of inhibition (ZOI) of only 6 mm against Pseudomonas, indicating not good inhibitory effect against this particular bacterium.

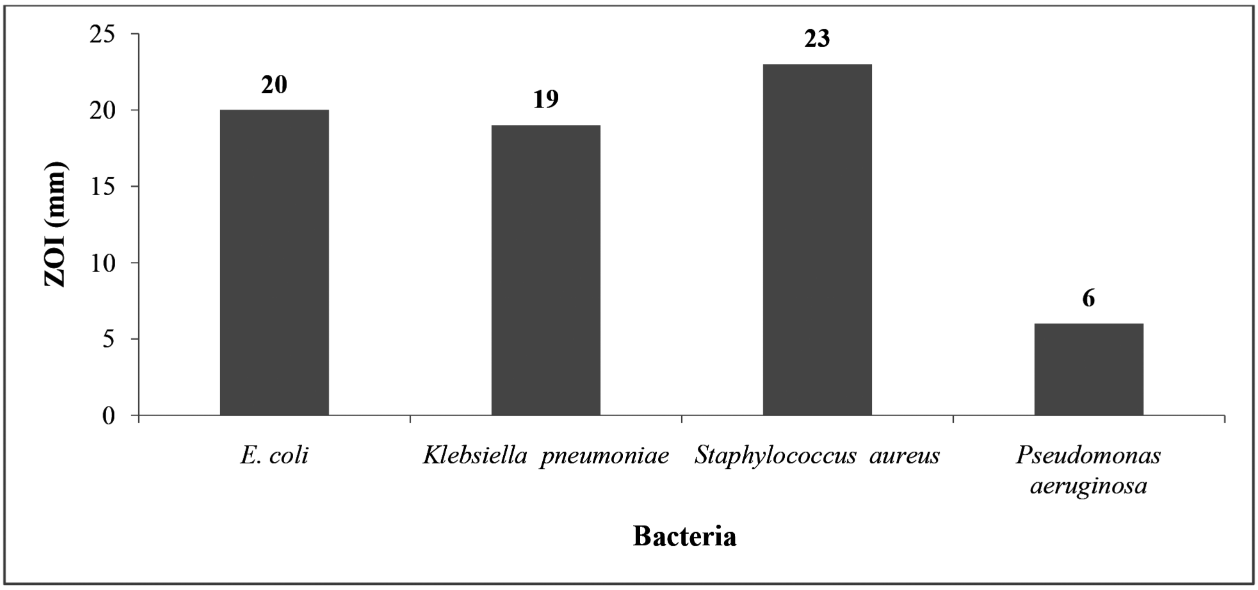

Figure 5: Zone of inhibition (ZOI) given by cardamom against tested bacteria.

4.3.5 Anti-bacterial activity of cinnamon

Cinnamon inhibited the growth of 3 isolates thus may be considered to have less Anti-bacterial activity.

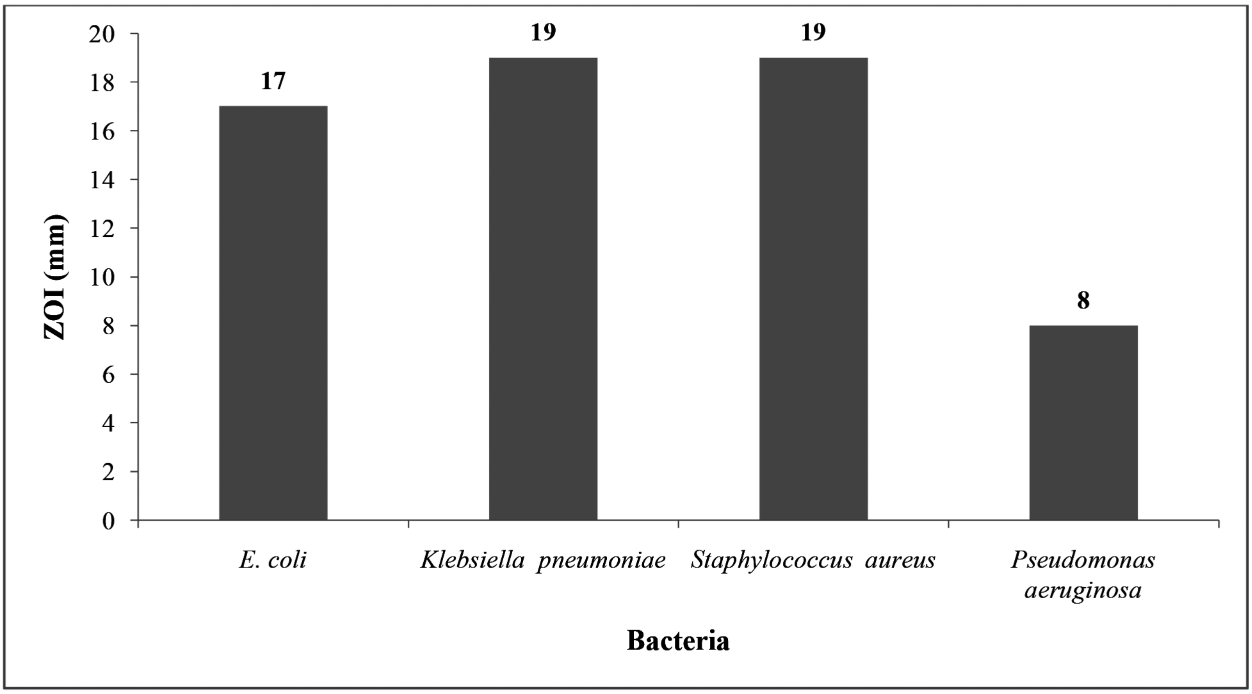

Figure 6: Zone of inhibition (ZOI) given by Cinnamon against tested bacteria.

4.3.6 Anti-bacterial activity of Fenugreek

Fenugreek didn't inhibit the growth of any four isolates thus may be considered to have weak Anti-bacterial activity.

Discussion

During our study, clove demonstrated remarkable effectiveness against all tested bacteria. The crude ethanol extracts of clove exhibited the highest antibacterial activity, effectively targeting bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Our findings are consistent with previous research by Agaoglu (2006) and Angihotri and Vaidya (1995), further supporting the well-established antimicrobial properties of clove. The active compound responsible for this antimicrobial action was identified as eugenol, as reported by Rana et al. (2011).

Ajowan also displayed significant antibacterial activity against the four tested bacteria, including *E. coli, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa*. The antimicrobial activity of ajowan was attributed to the presence of thymol, as reported by Maharjan (2008).

Among the spices studied, cinnamon showed remarkable effectiveness. The ethanol extracts of cinnamon exhibited strong antibacterial activity against all tested bacteria. The antimicrobial activity of cinnamon was attributed to the combined action of trans-cinnamaldehyde and eugenol, as reported by Dal Pozzo et al. (2012).

Similarly, cumin demonstrated notable antimicrobial properties. The ethanol extracts of cumin displayed potent antibacterial activity against all tested bacteria, with a significant effect on *E. coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The active compound responsible for this antimicrobial action was identified as cumin aldehyde (27.10%), as reported by Wongkattiya et al. (2019).

Cardamom also exhibited considerable effectiveness among the studied spices. Ethanol extracts of cardamom displayed antimicrobial activity against three tested bacteria, namely *E. coli, Klebsiella pneumoniae,* and *Staphylococcus aureus*. The active compound responsible for this antimicrobial action was identified as 1,8-cineole, as reported by Kapoor et al. (2008).

However, fenugreek displayed the weakest antimicrobial activity among the spices studied. The ethanol extracts of fenugreek showed limited effectiveness against all tested bacteria. The active compound responsible for this antimicrobial action was identified as diosgenin, as reported by Sharma et al. (2016).

It is worth noting that the difference between our results and those of previous researchers may be attributed to various factors such as test environments, methodologies, the quantity and age of spices used, and other variables. The antimicrobial activity of spices can also be influenced by farming practices, harvesting methods, storage conditions, and extraction procedures. Additionally, the volatility and poor solubility of certain essential oils can present challenges in diffusion and dilution within a microbiological medium.

CONCLUSION

In conclusion, the findings of the present study reveal that cumin, clove, and cinnamon extracts demonstrated significant antimicrobial activity. These spices, in the form of crude ethanol extracts, exhibited strong inhibitory effects against certain bacteria, indicating their potential as effective remedies against diseases caused by these microorganisms. Furthermore, the effectiveness of spices against spoilage bacteria suggests their potential as preservatives, offering an alternative to chemical additives that can compromise the quality of food and impact consumer health.

However, it is important to conduct additional studies to understand the specific mechanisms of action and evaluate the safety and effectiveness of these spice extracts in various contexts. Proper dosage, formulation, and application methods need to be explored to ensure their practical use in medicine and food industry.

Overall, this study underscores the valuable role of natural compounds found in spices and their potential as alternatives to traditional antimicrobial agents and chemical additives. Further exploration of these natural remedies may offer significant benefits in improving public health and food safety.

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