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Detection and determination of effect of antimicrobial efficacy of Cumin seeds (*Cuminum cyminum*) and Indian marigold flower (*Calendula officinalis*) against *Escherichia coli* and *Staphylococcus aureus* bacteria

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Abstract

Antimicrobial resistance is the most significant threat to global health. The emergence of resistant strains is accelerated by the misuse of antibiotics. Plant metabolites beneficial in preventing antibiotic resistance due to their potential to change resistance. Plants contain secondary metabolites which possess antimicrobial activities *in vitro* and are used as an effective antibiotic for the treatment of microbial diseases. *Calendula officinalis* Linn. is an annual and versatile herb which is widely used in homeopathic remedies. To treat infectious disease, all plant components (buds, leaves, and blooms) can be utilized dry or fresh. *Cuminum cyminum* is an aromatic herb widely used as a food and flavouring additive as well as often used in alternative medicine. Due to the presence of cuminaldehyde, cumin has great antimicrobial effects against both gram positive and negative bacteria. The major objective of this study is to determine the antimicrobial efficacy of cumin seeds and Indian marigold flower against *Escherichia coli* and *Staphylococcus aureus* bacterial strains. The phytochemicals are extracted using an ethanol and methanol maceration procedure, and their antibacterial capabilities are tested and assessed using ABST testing. The results reveal that the increasing the concentration of extract causes inhibition of bacterial growth. Therefore, 50 mg/ml and 100mg/ml concentration had less zone of inhibition (ZOI) compared with highest concentration which was 150 mg/ml. When the extract concentration was increased to 150 mg/ml, the bacterial growth inhibition zone increases significantly. When compared to ethanol extraction of cumin seeds and marigold flowers, the marigold flower showed more anti-bactericidal effects compared to the cumin seeds.

Keywords: *Calendula officinalis*, *Cuminum cyminum*, Antimicrobial resistance, *Escherichia coli*, *Staphylococcus aureus*

1. Introduction

The medicinal plants are extensively used as drugs to treat humans and animals to cure the various diseases since the beginning of human history. Therefore, medicinal plants are considered as the backbone of holistic medicine. Bioactive components of medicinal plants are widely used to cure various diseases.[1] Extracts of the whole plant or plant parts, including barks, stalks, blooms, leaves, roots, and fruits are utilized as potent natural medications to cure various diseases, specifically caused by microorganisms.[2]

Nowadays, antimicrobial resistance (AMR) is the biggest threats to global health. Each year, it causes around 2 million infections and 23,000 fatalities.[3] The ability of bacteria, parasites, viruses, and fungi to grow and proliferate in the presence of antimicrobial medications that are ordinarily active to defense them is known as antimicrobial resistance (AMR).[4]

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Plants contains phytochemicals constituents; tannins, alkaloids, flavonoids terpenoids and polyphenols. Plants utilizes these compounds as defense mechanisms against wide range of microorganisms.[5] Due to the current scenario, many ongoing research are conducted regarding plant based novel medications. Globally, 25% of prescribed drugs are plant based and more than 13000 plants are studies for last 5 years for medicinal purposes.[6]

Calendula officinalis commonly referred as 'Indian marigold' is a perennial herbaceous plant belongs to Asteraceae family. Marigolds are native to Europe, eastern and western Asia as well as in United states.[7] *C. officinalis* have angular hairy stem which can be growing up to 30-60 cm.[8] Also, it has long elliptical shape leaves. Marigold flowers are monoecious and has bright yellow to orange in color, but only dark orange color flowers have the medicinal value.[9] Flavonoids, tocopherols, terpenoids, phenolic compounds and carotenoids are the main phytoconstituents which possess pharmacological activities in *C. officinalis.*[10]

Marigold flowers are cultivated due to its nutritional, economical, and medicinal value. The petals extract has traditionally been used to dye natural fabrics (wool, silk).[11] Due to presences of saponins and essential oil, the extract was widely used for Cosmetic purposes.[12] In folk medicine, marigolds was used to treat wound healing, infectious diseases and burns since 12th century.[13] In wound healing process, the extract can stimulate the new tissues and blood vessels growth by topical application.[14]

Previous studies prove that marigold has hepatoprotective, hypoglycemic and antioxidant properties. According to laboratory experiment, *Calendula* petal extracts inhibits the human immunodeficiency virus (HIV), also neutralize the oxidative stress and liver damage followed by Aflatoxin.[14]

Figure 1. *Calendula officinalis* flower[14]

Figure 2. **A** – *C. officinalis* (young leaflet stage), **B** – *C. officinalis* (flower bud stage), **C** – *C. officinalis* (flowering stage)[15]

The past researchers have shown that under *in vitro* conditions, marigold flower and dried leaves have antibacterial properties against *Klebsiella species, Staphylococcus aureus*, and *Escherichia coli* in chloroform, methanolic and ethanol extracts.[16] Also, *in vitro* study confirms it has great inhibitory progression against skin microbiota.[17]

Marigold's antimicrobial activities were tested by antioxidant and UV induced DNA damage prevention activity.[18] DPPH and FRAP phytochemical analysis endorsed that *C. officinalis* extract has antimicrobial and antioxidant action.[19]

Cuminum cyminum generally known as 'cumin seeds' which is an aromatic herb belongs to the family Apiaceae.[20] *C. cyminum* are originated from Egypt and Turkey but due to their

special aromatic effect, the dried cumin seeds are broadly used in Asian, middle Eastern and Latin American cuisine.[21]The plant can grow up 25 cm, and has tiny whitish or pink color, umbellate formation flowers and 3-6 mm elongated, striped pattern seeds.[22]

Figure 3. Life cycle of the *C. cyminum*[23]

Cumin seeds has been in use as an alternative medicine for a long time, to cure chronic diarrhea, epilepsy, and jaundice.[24] It is considered as a carminative, antispasmodic and astringent also, cumin oil inhibits numerous pathogenic *Candida* species due to its antifungal properties.[25]

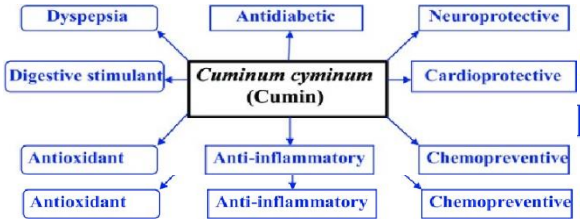


Figure 4. Medicinal values of Cuminum cyminum[26]

The plant consists of cuminaldehyde, tannin, and cymene compounds, it has potential properties in aspects such as antimicrobial, antidiabetic and hypolipidemic.[27] Therefore, due to their pharmacological characteristics, studies on *C. cyminum* roots, stems, leaves, and flowers have garnered interest.[28] Previous studies confirm that cumin extract inhibits the growth of various pathogens (*E. coli, S. aureus, Salmonella* species, and *Aspergillus niger*).[29] Antimicrobial potential of cuminaldehyde counteracts gram positive and negative bacteria respectively.[30] Cumin has been shown to inhibit the production of biofilms in *Streptococcus mutans* and *Streptococcus pyogenes.*[31] Cumin has an exceptional antibiofilm and quorum sensing inhibitory activities against gram-negative bacterial pathogens.[32]

The researchers find, plant origin antimicrobial compounds have great therapeutic potential against wide range of microorganism and have less side effects compared to synthetic drugs.[33] Therefore, plant base novel medications are effective and safe for human and animal consumption.

Gram positive and negative bacteria respectively, *Staphylococcus aureus* and *Escherichia coli*, which contribute for foodborne diseases and have increased the morbidity and mortality rate globally.[34]

Staphylococcus aureus is responsible for methicillin-resistant *Staphylococcus aureus* (MRSA) nosocomial disease, currently resistance for all type antibiotics. Due to the evolution of organisms carrying extended spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases, *Escherichia coli* develops inherent resistance to several types of β-lactamase.[35]

Both bacteria develop their resistance to antimicrobials followed by these mechanisms such as restricting acceptance of drugs, modification of drugs, enzymatic degradation of drugs and active efflux of the drugs.[36]

Due to misuse and repeated usage of antibiotics, the bacterial pathogens become resistance. Also, high consumption of antibiotics caused to escalate the side effects. Inhibiting efflux pumps and eliminating plasmids cause to increase the medicinal efficiency of antibiotics, that could help to decrease the foodborne pathogens antibiotic resistance throughout the food chain.[37]

2. Methodology

2.1 Collection of medicinal plants. Fresh healthy and disease-free *C. officinalis* flowers and *Cuminum cyminum* seeds were respectively collected from home gardens and local market of Matara district, Sri Lanka.

2.2 Preparation of plant Extracts. C. officinalis flowers and *C. cyminum* seeds were thoroughly washed with tap water then distilled water and the surface was sterilized with 70% ethanol. The cumin seeds and separated marigold flower petals and sepals were shade dried for three days. The dried plant materials were pulverized to a fine powder discretely by using mechanical grinder and passed-through a 0.5 mm mech sieve. About 200 g of marigold flower powder and 250 g of cumin seeds powder were gained after pulverization. The plant powder materials were stored in separate airtight containers for further investigation.

2.3 Ethanolic extraction of Calendula officinalis and Cuminum cyminum. Ethanolic extraction was carried out using marigold and cumin powder with 70% and 80% of ethanol respectively according to 1:10 (w/v) ratio. 2g of dried marigold powder was extracted with the combination of 14.73 ml ethanol and 5.27 ml of distilled water. 2 g of cumin powder was added to 80% of ethanol (20 ml) with the combination of 16.84 ml ethanol and 3.16 ml of distilled water.

2.4 Methanol extraction of *Calendula officinalis* and *Cuminum cyminum.* Methanolic extraction was carried out using marigold and cumin powder with 70% and 80% of methanol respectively according to 1:10 (w/v) ratio. Both ethanolic and methanolic plant extraction samples were kept in roller mixer at 27°C for 24 hrs. The macerate was filtered with Whatman no 01-filter paper, and the solvent was kept into fume hood for 48 hrs for completely evaporation. Finally, the extracted crude was reconstituted in DMSO to get stock concentration of 200 mg/ml.

2.5 Bacterial strains. Methanol and ethanol extracts of *C. officinalis* and *C. cyminum* were tested discretely against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) bacterial strains.

2.6 Inoculum preparation. 100 µL of *Escherichia coli* and *Staphylococcus aureus* were inoculated into 5 ml of LB broth in separate falcon tubes and incubated at 37°C for 24 hours. The bacterial suspension turbidity was adjusted according to 0.5 McFarland standard by using a Wickerham card in the presence of the sufficient light.

2.7 Antibiotic Sensitivity Testing. The Mueller Hinton agar (MHA) was prepared and poured into petri plates and allowed to solidify for few minutes at aseptic condition. The bacterial inoculum was swabbed by rotating the plate around for uniform distribution on the entire surface. Finally, the edge of the of the agar plates were swabbed by using sterilized cotton swab. Four wells were prepared by using sterile 1000 µL pipette tips. 50µl of reconstituted plant sample concentrations (50 and 100 mg/mL) and DMSO and Gentamicin (1 mg/mL) were used as negative and positive controls, respectively. Then solvents were introduced into separate respective wells. The plates were incubated for 24 hours at 37°C in an upright position. The diameter of the zone of inhibition was measured to the nearest millimeter to determine antibacterial activity. The experiment was done in triplicate and the average values were calculated.

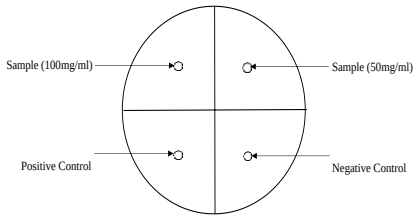
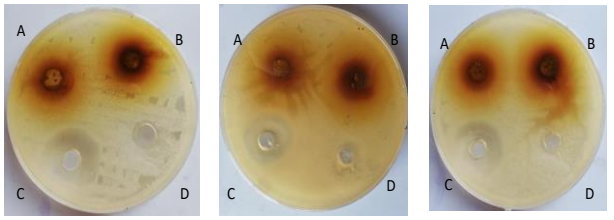


Figure 5. Outline of the petri plate for well diffusion

2.8 Statistical analysis. Results were observed and recorded in triplicates and the data were analyzed statistically to evaluate correlation among variables (antibiotic profiles) using the two- way ANOVA test using the GraphPad Prism software (version 9.1.0). The students t- test was used to evaluate the extraction efficacy between the solvents used. The statistical significance was denoted as p value ≤ 0.05 for a 95% confidence interval.

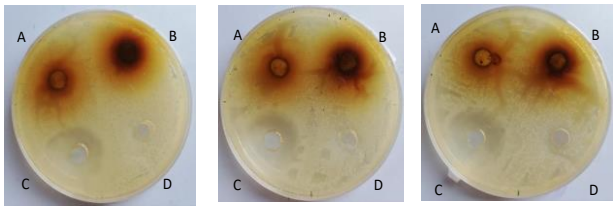
3. Results

3.1 Antibacterial Susceptibility Testing (ABST) for ethanolic extracts of Indian marigold



ABST results for *S. aureus*

R1 **R2** **R3**



ABST results for *E. coli*

R1 **R2** **R3**

Figure 6. The ZOIs produced by ethanolic extracts of Indian marigold against *E. coli* and *S. aureus*; (A)= 100mg/mL plant extract, (B)= 150mg/mL plant extract, (C)= positive control and (D)= negative control, (R= replicate).

Table 1. ZOIs measured for ethanolic extracts of Indian marigold

	100mg/mL (mm)	150mg/mL (mm)	Positive (mm)	Negative (mm)
E. coli	17.667 ± 0.577	21.000 ± 2.646	24.666 ± 1.527	-
S. aureus	18.000 ± 1.732	19.333 ± 1.55	26.333±1 .154	-

Highest ZOI was observed in 150mg/mL against *E. coli* while the lowest ZOI was in 100mg/mL against the same strain.

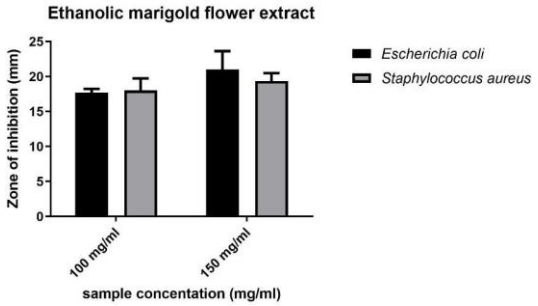


Figure 7. The antibacterial properties of 100 mg/mL and 150 mg/mL ethanolic extract of Indian marigold depicted using well diffusion test. *E. coli* is represented by the black color bars and *S. aureus* is represented by grey color bars. The bars show mean ± SD of the inhibition zones for three replicates.

Table 2. Two-way ANOVA analysis for ethanolic extract of Marigold flower

ANOVA Table	SS	DF	MS	F	P
Interaction	3.00	1	3.00	1.029	0.3402
Concentration	16.33	1	16.33	5.600	0.0455
Bacteria	1.33	1	1.33	0.4571	0.5180
Residual	23.33	8	23.33		

According to the two-way ANOVA analysis, P value for interaction has 0.34 which is higher than the 0.05. Therefore, it is not significant. Also, P value for bacteria is 0.5 which is higher than the 0.05. Therefore, it is not significant. The extract concentration is 0.04 value and it was lower than the p value, and it is significant. Therefore, increasing the concentration, the main effects are observed and they are contributing to the main effects.

3.2 Antibacterial Susceptibility Testing (ABST) for methanolic extracts of Indian marigold.

Table 3. ZOIs measured for methanolic extracts of *Indian marigold*

	50mg/mL (mm)	100mg/mL (mm)	Positive (mm)	Negative (mm)
E. coli	12.100± 0.656	12.833± 0.764	23.000± 0.00	-
S. aureus	10.000± 1.00	17.500± 0.500	20.00±0.00	-

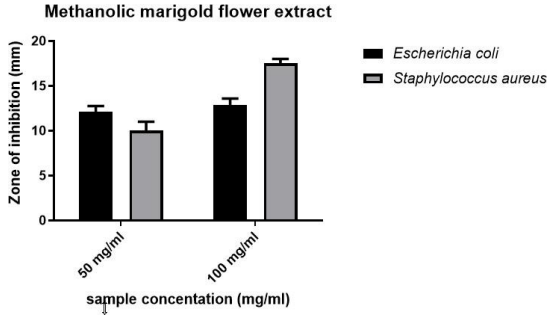


Figure 8. The antibacterial properties of 50 mg/mL and 100 mg/mL methanolic extract of Indian marigold depicted using well diffusion test. *E. coli* is represented by the black color bars and *S. aureus* is represented by grey color bars. The bars show mean ± SD of the inhibition zones for three replicates.

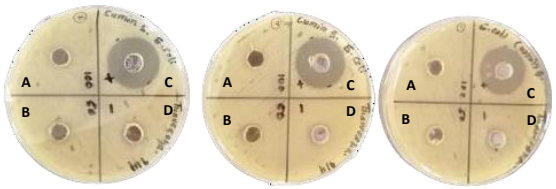
Table 4. Two-way ANOVA analysis for methanolic extract of Marigold flower

ANOVA table	SS	DF	MS	F	P
Interaction	34.34	1	34.34	60.69	0.0001
Concentration	50.84	1	50.84	89.85	0.0001
Bacteria	4.941	1	4.941	8.732	0.0183
Residual	4.527	8	4.527		

According to the two-way ANOVA analysis, P value for interaction has 0.0001 which is lower than the 0.05. Therefore, it is significant. Also, P value for bacteria has 0.0183 which is also, lower than the 0.05. Therefore, it is also significant. The concentration has 0.0001 value and it was lower than the p value, and it is significant. Therefore, all the three facts are significantly different and they are contributing to the main effects.

3.3 Antibacterial Susceptibility Testing (ABST) for ethanolic extracts of cumin seeds

ABST results for *E. coli*



ABST results for *S. aureus*

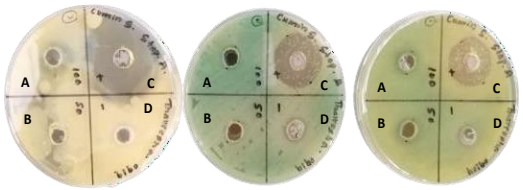


Figure 9. The ZOIs produced by ethanolic extracts of cumin seeds against *E. coli* and *S. aureus*; (A)= 100mg/mL plant extract, (B)= 50mg/mL plant extract, (C)= positive control and (D)= negative control, (R= replicate).

Table 6. ZOIs measured for ethanolic extracts of cumin seeds

	50mg/mL (mm)	100mg/mL (mm)	Positive (mm)	Negative (mm)
E. coli	9.900 ± 0.794	10.667±1.155	26.000±1.732	-
S. aureus	11.000± 0.000	11.000±0.000	20.333±1.000	-

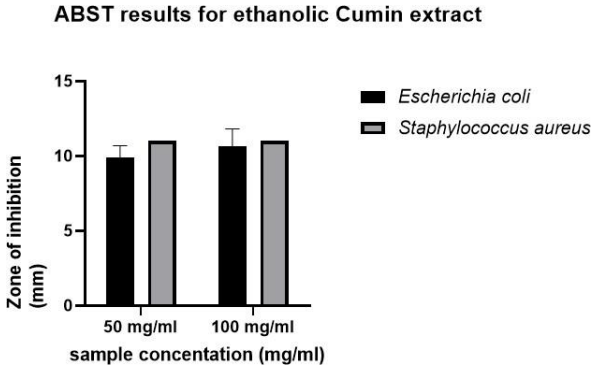


Figure 11. The antibacterial properties of 50 mg/mL and 100 mg/mL ethanolic extract of cumin seeds depicted using well diffusion test. *E. coli* is represented by the black color bars and *S. aureus* is represented by grey color bars. The bars show mean ± SD of the inhibition zones for three replicates.

Table 7. Two-way ANOVA analysis for ethanolic extract of cumin seeds

ANOVA table	SS	DF	MS	F	P
Interaction	0.4408	1	0.4408	15.17	0.3710
Concentration	0.4408	1	0.4408	1.567	0.3710
Bacteria	1.541	1	1.541	4.648	0.1144
Residual	3.927	8	3.927		

According to the two-way ANOVA analysis, P value for interaction has 0.3710 which is higher than the 0.05. Therefore, anti-bacteria effect of this concentration it is not significant. Also, P value for bacteria has 0.1144 which is higher than the 0.05. Therefore, it is not significant. P value of the concentration of the sample is >0.05. Hence, there is no significant difference between sample concentration and the antibacterial activity against the bacterial strains. However, all the three facts are not significant and they are not contributing to the main effects.

3.4 Antibacterial Susceptibility Testing (ABST) for methanolic extracts of cumin seeds

Table 5. ZOIs measured for Methanolic extracts of cumin seeds

	50mg/mL (mm)	100mg/mL (mm)	Positive (mm)	Negative (mm)
E. coli	16.033±0.850	16.867 ± 0.551	20.05 ± 0.7	-
S. aureus	18.500 ± 1.000	15.600 ± 0.854	21.5± 3.5	-

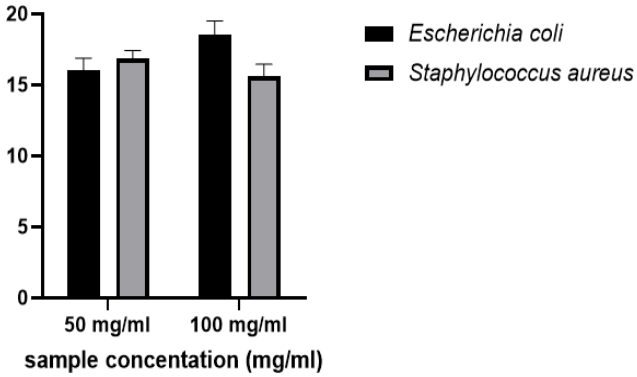


Figure 10. The antibacterial properties of 50 mg/mL and 100 mg/mL methanolic extract of cumin seeds depicted using well diffusion test. *E. coli* is represented by the black color bars and *S. aureus* is represented by grey color bars. The bars show mean ± SD of the inhibition zones for three replicates.

Table 8. Two-way ANOVA analysis for methanolic extract of cumin seeds

ANOVA table	SS	DF	MS	F	P
Interaction	10.45	1	10.45	15.17	0.0046
Concentration	1.080	1	1.080	1.567	0.2460
Bacteria	3.203	1	3.203	4.648	0.0632
Residual	5.513	8	5.513		

According to the two-way ANOVA analysis, P value for interaction has 0.0046 which is lower than the 0.05. Therefore, it is significant. Also, P value for Concentration has 0.2460 which is higher than the 0.05. Therefore, it is not significant. The Bacteria has 0.0632 value and it was not lower than the p value, and it is not significant.

4. Discussion

The selective pressure that leads to the emergence of antibiotic resistance in microorganisms is caused by human overuse of antibiotics. Emerging multidrug resistant pathogens, often known as 'ESKAPE' species represent a severe threat to global health today.[38] Medicinal plants contain phytochemical compounds which naturally associated with fighting against microbial resistance and also have antimicrobial properties. Therefore, researchers are interested in plant-based medications as an undisputable substitution for antibiotics due to high efficacy to antimicrobial activity with less side effects as well as low cost.[39]

The plant age and part are important parameters for extraction procedure. Recently matured plants are ideal for researchers. If the plant part is too old, the phytochemical compounds have less effective. When it comes to alkaloids, older plants contain significantly fewer than younger plants. Phytochemical compounds' efficacy varies depending on the stage of plant development.

Also, dried samples are better than the fresh plant samples. Fresh samples are caused to quick degradation due to water content of the sample. When the particle size is reduced, the solubility and diffusion increase, which leads to an increase in the extraction rate. As a result, the powdered sample has a greater surface area contact with the extraction solvent. Particles smaller than 0.5mm should be used for better extraction. The extraction duration, temperature, growth regions and the quality of the seeds can affect the differences of the zone of inhibition.[40] In phytochemical extractions, three types of solvents are used: polar, intermediate-polar, and non-polar, and the solvents are chosen depending on the polarity of the solute. Ethanol is a polar molecule that may dissolve in polar and non-polar solvents. It's ideal for polyphenol extraction and is safe for human consumption. Methanol has been found to be more effective at extracting polyphenols with lower molecular weights. In ethanolic extraction, a higher extraction yield was observed for *Calendula officinalis* which is 9.5% compared to the *Cuminum cyminum* which is 9.0%.

For ZOI, drug solubility, concentration, media composition and thickness, temperature, atmosphere and incubation time are all influenced. Each experiment was carried out at least three times. The results are expressed as Mean and SD.

The results revealed that each of the cumin extract concentrations tested had antibacterial action against both gram positive and gram-negative microorganisms. Comparing the results of methanolic extractions, cumin seeds showed the maximum ZOI against S. aureus which was 18.500 ± 1.000 at 50 mg/mL while showed, 15.600± 0.854mm for 100mg/ml. For *E. coli* bacteria, obtained highest ZOI observed at the concentration of 100mg/ml (16.867 ± 0.551) compared to concentration of 50mg/ml (16.033 ± 0.850). The overall results of the methanolic extract, the highest anti-bacterial activity was observed at 50mg/ml for gram positive bacteria. According to Santajit *et al*.[38], methanolic extract of cumin for *Escherichia coli* the inhibition zones were observed at 250 mg/ml which is 16.67±0.47. Compared to this study, our results obtained comparatively high values at low concentration compared to Sheikh's study.

Also, according to the Bouhenni's study, at the concentration of 100 mg/ml, *S. aureus* and *E. coli* bacterial strains showed ZOI with diameters of 21 mm± 0.333 and 12 mm ± 0.66 respectively.[41] High ZOI dimeters were found in the Bouhenni's investigation for *S. aureus*. In contrast, our investigation found 15.600 ± 0.854mm for *S. aureus*. Masood and Tariq reported that cumin extract inhibited the *S. aureus* and *E. coli* growth with the diameters 8.9±5.6 and 23.8±1.2 respectively.[42] Soniya's study found that methanol extracts of *C. cyminum* inhibited *Bacillus subtilis*, *E. coli*, and *Proteus sp*. with the maximum diameter of zones of inhibition.[43]

Comparing the results of ethanolic extracts, *S. aureus* showed high ZOIs at the concentration of both 100 mg/ml and 50 mg/ml (11.000 ± 0.000). Cumin ethanolic extract, the *E. coli* showed, 9.900 ± 0.794mm and 10.667 ± 1.155mm for 50 and 100mg/ml respectively. According to the study done by Mostafa et al[45], *C. cyminum* extract inhibit the growth of *S. aureus* at the concentration of 10 mg/ml which was 9.5 ± 0.74 but at the same concentration *E. coli* showed 0.0 ± 0.0, which means at the concentration of 10 mg/ml, *E. coli* was totally not resistant.[44] Compared to our project, Mostafa *et al*[45]. got a comparatively high ZOI for *S. aureus* at concentration of 10 mg/ml.[45] Based on the Sheikh *et al*.[41] 15.67±0.47 inhibition zones were recorded at 250 mg/ml against *E. coli*.[38] Cuminaldehyde is the active component in cumin extract, which has great antimicrobial effects against both gram positive and negative bacteria. Cuminaldehyde alters the bacterial cell's outer layer, preventing ion transfer into and out of the cell. In the end, this mechanism disrupts the action of bacterial enzymes.[46]

In this study reveals that marigold flower ethanolic extraction showed highest ZOI for gram-positive bacteria at concentration 150 mg/ml (19.333 ± 1.55mm) compared to 100 mg/ml (18.000 ± 1.732). For gram negative bacteria, higher concentrations are required for inhibit the growth of the bacteria. According to that, the maximum ZOI was measured at 150 mg/ml (21.000 ± 2.646mm) also, at 100 mg/ml 17.667 ± 0.577 mm ZOI were observed. Ethanol extract of marigold showed 14 and 18 mm of ZOI against *Staphylococcus aureus* and *E. coli* respectively at 50 mg/ml.[47]

Previous studies have shown that methanolic extracts of the marigold flower have antibacterial activity against *Klebsiella* species, *Staphylococcus aureus*, and *Escherichia coli*.[48] Comparing the results of methanolic extracts, *S. aureus* showed highest ZOI at 100 mg/ml (17.500 ± 0.500mm) compared to 50 mg/ml (10.000 ± 1.00mm). In *E. coli* samples, highest ZOI was observed at 100mg/ml (12.833 ± 0.764mm) compared to 50mg/ml (12.100 ± 0.656mm). Efstratiou *et al*.[11] reported that at 300 mg/ml in methanolic extract, *E. coli* showed ZOI 21 ± 2mm while *S. aureus* got ZOI 18 ± 2mm.[11]

The results reveal that when increasing concentration cause to inhibition of the bacteria. Therefore, 50 mg/ml and 100mg/ml concentration had less ZOI compared with highest concentration which was 150 mg/ml. When the extract concentration was increased to 150 mg/ml, the bacterial growth inhibition zone increases significantly. When compared to ethanol extraction of cumin seeds and marigold flowers, the marigold flower showed more bactericidal effects compared to the cumin seeds.

When comparing both stains, gram negative bacteria showed highest inhibitory properties at the highest concentrations. This could be due to Gram-negative bacteria having an additional outer membrane made of lipopolysaccharide, which makes them impervious to lipophilic, but Gram-positive bacteria only have an outer peptidoglycan layer, which is ineffective as a permeability barrier.

The negative control was DMSO, which had no antibacterial effect on the microorganisms examined. Gentamicin, on the other hand, demonstrated an antibacterial impact on the microorganisms tested when used as a positive control antibiotic. It inhibits protein synthesis and causes death in susceptible bacteria by attaching to the 30S component of the bacterial ribosome.

Considering results of ANOVA analysis for ethanolic extraction, the marigold flower has 0.04 value for concentration which is significant. For, cumin seeds P value of the concentration of the sample was 0.3710 which is higher than the p value (0.05). Therefore, there is no significant difference between sample concentration and the antibacterial activity against the bacterial strains in cumin seed concentration. According to the ANOVA analysis of methanolic extraction, the cumin seeds have a P value of 0.2460 for concentration which is more than 0.05. As a result, it is not significant. For marigold flower, the concentration has 0.0183 value and it was lower than the p value, and it is significant.

Conclusion

C. officinalis and *C. cyminum have* significant antimicrobial activities against both gram positive and negative bacteria. The research findings can be used to development of new plant-based drugs which has fewer side effects as well as highly effective for infectious diseases. As a result, further pharmacological and clinical research is needed to better understand the mode of action of medicinal plants and microbes, as well as the efficiency of herbal drugs in treating specific bacterial infections.

Many plant-derived medications are undergoing clinical trials, and at least 100 compounds are in the preclinical stage of research. Natural products are used to develop drugs for cancer and infections, which are the two most common treatment fields.

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