QUEST
A Peer Reviewed Research Journal
ISSN : 2583-9748 (Online)

https://www.questjournal.in

Assessment of Antibacterial Efficacy of *Trachyspermum Ammi* and *Piper Nigrum*

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Received : December, 2023
Accepted : January, 2024
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Abstract: *Spices have a significant role in maintaining the wellbeing of human kind. Trachyspermum ammi and Piper nigrum has the potency to be used as an alternative source as antibacterials. They stand against various bacterial pathogens Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and has been employed in traditional medicine for centuries. It was found to have competitive effects compared to antibiotics Ampicillin, Gentamicin, Norfloxacin, Streptomycin. Evaluation of antibacterial activity of methanolic, ethanolic, aqueous extract prepared from powdered seed of Trachyspermum ammi and Piper nigrum determined by disc diffusion method in Muller Hinton agar against bacteria. 25µg/ml seed extract was tested against the bacteria and compared with antibiotics 10µg/disc. Zone of inhibition was measured and calculated. The result showed that Trachyspermum ammi and Piper nigrum extract had good antibacterial activity.*

Keywords: *Trachyspermum ammi, Piper nigrum, Klebsiella pneumoniae, Ampicillin, Gentamicin, Norfloxacin, Streptomycin, Antibacterial activity.*

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Introduction:

India, which is also known as the 'land of spices' blessed with a varied climate, is one of the largest producer, consumer and exporter of spices. India grows over fifty spices out of eighty-six grown worldwide. Spices has an important place in common man's life primarily used for flavoring, colouring or preserving food and also being used for medicinal purpose for prevention of diseases. There is no food in which spices are not being used and that is why Indian food and its traditional recipes have been considered the best for centuries (Alagusundaram and Kumaravel, 2014).

The production of spices increases every year and it plays significant role in the economy of our country. Presently its production is of 5.47 million tons because of diversified use and higher demands. Spices are being widely used in indigenous medicine pharmaceuticals, aromatherapy, preservatives, beverages, perfumes, natural colours, dental

preparations, botanical pesticides and cosmetics (Bhagya *et.al.,* 2017).

The medical practice using spices was first seen in Ancient Egypt. The ancient Egyptians utilized spices as a part of therapy. The Ebers Papyrus, which was written in Egypt about 1500 BC, mentioned the coriander, fennel, garlic, mint, poppy, onion, peppermint for medical treatments (Grevetti and Louis, 2016). India is a treasure of medicinal plants and spices from ancient period and its importance has been mentioned in an ancient scholastic book such as Rigveda, Atharvaveda, Charka, Sushruta etc.

Spices contain phytochemicals like Terpenes, Monoterpenes, Tetra terpenes (carotenoids), Sesquiterpenes, Terpene derivatives, Cinnamic acid, Thiols, Sulfides etc., antioxidants like Rosemary, Cinnamon and bioactive molecules like Tannin, Vitamins, Sulfur mixing etc., has the antioxidants and antimicrobial properties that have been used in reducing the rate of many diseases (Bhagya *et.al.,* 2017).

In present study, an attempt has been made to enrich the knowledge of antibacterial activity (AMA) of ***Trachyspermum ammi*** and ***Piper nigrum*** seed extract.

Trachyspermum ammi L. belongs to family Apiaceae. It is a small, erect, annual, herbaceous plant with leafy branched stems and feathers like leaves 12.5cm long bearing 4-12 ray flower heads. The fruits are greyish brown coloured, minute and egg shaped. Ajwain is considered as a medicinally valued seed spice. These seeds are used in flavoring of food, preservatives and medicines and its essential oils is used for perfumery. Ajwain has been used as an antibacteria, for the treatment of gastrointestinal problems, lack of appetite, bronchial problems and is a common household remedy for the stomach disorders and colic pains (Sharma *et.al*., 2018).

Piper nigrum commonly known as Kali mirch is a native of South Asia and Southeast Asia and is primarily grown in China, Vietnam, Indonesia, Brazil, Malaysia and India. In India, it is mostly cultivated in Kerala, Karnataka, Tamil Nadu, Maharashtra, Northeastern States and Andaman and Nicobar Islands.

Piper nigrum belongs to family Piperaceae. It is a perennial, flowering vine, growing on supporting trees and poles, with a long leaf of about 5-10 cm bearing flowers which are small in size. Black pepper is produced from the green unripe drupes of pepper plant (Harrison, 2016). The black peppercorns used in different types of foods and medicines and thus also known as “The king of Spices”. Peppercorn has been used in Ayurvedic and Unani system of medicines from ancient times. Pepper contains Piperine which exhibit effects as analgesics, antispasmodic, antidepressants, antibacterial, antifungal etc. (Ahmad and Damanhouri, 2014). The antibacterial activities of *Trachyspermum ammi* L. and *Piper nigrum* have not been scientifically analyzed. Hence the present study has been designed to evaluate the same.

To observe the anti-bacterial activity of plants, different types of bacterial strains like *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae* and different antibiotics like Ampicillin, Gentamicin, Norfloxacin, Streptomycin were chosen. Antibiotics were selected as a reference compound and the disc of antibiotics was used for a susceptibility test to check the zone of inhibition (ZOI).

Materials and Methods:

1. Procurement of seeds: Ajwain (*Trachyspermum ammi*) seeds and black pepper (*Piper nigrum L.*) seeds were purchased from local market of Patna.

2. Preparation: Seeds of *Trachyspermum ammi* and *Piper nigrum* were washed, sterilized, air dried and grinded to fine powder with a mortar and pestle.

3. Extraction: Aqueous, ethanol, and methanol extract of *Trachyspermum ammi* and *Piper nigrum* was prepared by dissolving 25 grams of its seed powder in 100ml of distilled water, ethanol and methanol respectively.

Ultra sonication assisted extraction:- After 48 hours, further extraction was done by ultra-sonication process at 25^0C for 5 hours.

4. Collection of bacteria:

Gram positive bacteria:- *S. aureus, S. epidermidis, Bacillus subtilis* and

Gram negative bacteria:- *Escherichia coli, klebsiella pneumonia* were collected from SRL Diagnostics laboratory, Fraser Road Patna due to their clinical and pharmacological significance.

5. Preparation of bacterial inoculums: The test organism of each strain was sub cultured on nutrient agar medium by incubating at 37^0C for 24hours and from this fresh culture, standard size inoculum was prepared.

6. Selection of antibiotics: Four antibiotics were used to compare their antibacterial effects with those of spice extracts.

Four Antibiotics were: Ampicillin (10mg), Gentamicin (10mg), Norfloxacin (10mg), and Streptomycin (10mg).

7. Preparation of MHA media: For the preparation of Muller Hinton Agar (MHA) MEDIA, 12grams of MHA (HIMEDIA, India) was dissolved in 400ml distilled water in a conical flask of 500ml.

The conical flasks were heated to boiling to dissolve the medium completely, sterilized by autoclaving at 15lbs pressure and 121^0C for 15 minutes and cool it to 45-50^0C and then media was poured in 20 different petri dishes and left for 10 minutes for solidification of MHA media in laminar air flow (LAF).

8. Antibacterial assay: Antibacterial activities of different extracts were determined by disc diffusion method (*Balouiri* et.al*.,* 2016).

The 5mm of disc was made of whatmann filter paper were impregnated in the methanolic, ethanolic and aqueous extracts of spices and left to be dried for 10 minutes.

Bacterial inoculum was spread on the surface of each MHA plate and impregnated discs were placed in the inoculated agar plate by sterile forceps in such a way that no overlapping of the zones could occur.

All treated petri plates were immediately placed in incubator at 37˚C for 24 hours.

After 24 hours incubation presence or absence of inhibition zone surrounding the discs were measured in millimeter (mm) and used as a measure of antibacterial activity.

9. Comparison with the standard antibiotics: Antibacterial activity of all selected antibiotics discs was tested by disc diffusion method.

The zones of inhibition were measured and then compared with the zone of inhibition of different extracts of spices.

Results:

A. Anti- bacterial Screening: The antibacterial activity of methanolic, ethanolic and aqueous extract of *Trachyspermum ammi* seed (ajwain) and *Piper nigrum* seed (black pepper) against bacteria has been assessed and compared its zone of inhibition with antibiotics as a reference. Primarily based on whether there was inhibition or lack of inhibition in bacterial growth and on which extract of seed have high potency against bacterial growth are tentatively tabulated and compared with the potential of seed extract and antibiotic used.

Methanolic extract of *Trachyspermum ammi* (ajwain) showed highest antibacterial potency against different strain of bacteria followed by ethanolic extract and aqueous extract.

Methanolic extract of *Piper nigrum* (black pepper) showed more antibacterial potency against gram positive bacteria with zone of inhibition of 16mm in *Bacillus subtilis* and ethanolic extract showed more antibacterial potency against gram negative bacteria with zone of inhibition of 14mm in *Klebsiella pneumoniae*.

Table 1. Anti-bacterial activity of methanolic, ethanolic and aqueous extract of, *Trachyspermum ammi* (T), *Piper nigrum* (P) and antibiotics against different strain of bacteria

S. N.	Bacteria	Antibiotics (10µg/ disc)				Methanolic Extract (500µg /disc)		Ethanoic Extract (500µg/ disc)		Aqueous Extract (500µg/ disc)	
		A	G	N	S	T	P	T	P	T	P
1.	*E. coli*	+	+	+	+	+	+	+	+	+	+
2.	*Klebsiella Pneumonia*	+	+	+	+	+	+	+	+	_	_
3.	*S. aureus*	_	+	+	+	+	+	+	+	+	+
4.	*Bacillus Subtilis*	_	+	+	+	+	+	+	+	_	_
5.	*S. epidermidis*	+	+	+	+	+	+	+	+	+	+

Antibiotics
A- Ampicillin.
G- Gentamicin.
N- Norfloxacin.
S- Streptomycin.

Methanolic, Ethanolic and Aqueous extracts
T - *Trachyspermum ammi* (ajwain).
P - *Piper nigrum* (black pepper)

In the table 1, the positive (+) sign indicates that it can inhibit the growth of bacteria and the zone of inhibition was found. The negative (-) sign indicates that it cannot inhibited the growth of bacteria and zone of inhibition was not found.

B. ZONE OF INHIBITION (ZOI) OF ANTIBIOTICS:

Ampicillin: The maximum zone of inhibition (ZOI) was 8mm in *E.coli* 7mm in *Klebsiella pneumoniae* and *S. epidermidis*. *S. aureus* and *B. subtilis* did not show any ZOI.

Gentamicin: The maximum zone of inhibition (ZOI) was 20mm in *S. aureus* and minimum (ZOI) was 13mm in *B. subtilis*.

Norfloxacin: The maximum (ZOI) was 32 mm in *B. subtilis* and no (ZOI) found against *S. epidermidis*.

Streptomycin: The maximum ZOI was 19mm found in *S. aureus* followed by *Klebsiella pneumonia, S. epidermidis* and *E. coli* with ZOI 15mm and least anti-bacterial activity was found in *B. subtilis* with ZOI 7mm.

Table 2. Anti-bacterial Activity of Antibiotics –ZOI (mm)

BACTERIA		ANTIBIOTICS (10µg/disc)			
		Ampicillin	Gentamicin	Norfloxacin	Streptomycin
1.	*E. coli*	8mm	16mm	18mm	15mm
2.	*Klebsiella Pneumonia*	7mm	15mm	18mm	15mm
3.	*S. aureus*	NA	20mm	22mm	19mm
4.	*B. subtilis*	NA	13mm	32mm	7mm
5.	*S. epidermidis*	7mm	18mm	NA	15mm

Norfloxacin showed best anti-bacterial activity, Gentamicin and Streptomycin showed temperate and Ampicillin showed the least anti-bacterial activity (Table 2).

(C) ZONE OF INHIBITION (ZOI) OF *Trachyspermum ammi* Seed (Ajwain) EXTRACT:

Methanolic Extract: methanolic extract of ajwain seed showed strong anti-bacterial activity against all strain of bacteria. Maximum 25mm ZOI against *Klebsiella pneumoniae* and minimum 15mm ZOI against *S. epidermidis*.

Ethanolic Extract: ethanolic extract of ajwain seed showed moderate antibacterial action. 15 mm ZOI against *S. aureus* and *B. subtilis* 12.5mm, 11mm, 8mm ZOI against *Klebsiella pneumoniae, S. epidermidis, E. coli* respectively.

Aqueous Extract: aqueous extract of ajwain seeds showed mild action. 11mm ZOI against *S. aureus* and *B. subtilis* and 7mm ZOI against *S. epidermidis* and *E. coil* and no antibacterial action against *Klebsiella pneumoniae.*

Table 3. Anti -bacterial activity of *Trachyspermum ammi* seed (Ajwain) extracts – ZOI (mm)

BACTERIA		METHANOLIC EXTRACT (500µg / disc)	ETNANOLIC EXTRACT (500µg/disc)	AQUEOUS EXTRACT (500µg/disc)
1.	*E. coli*	22mm	8mm	7mm
2.	*Klebsiella Pneumonia*	25mm	12.5mm	NA
3.	*S. aureus*	23mm	15mm	11mm
4.	*B. subtilis*	15mm	15mm	11mm
5.	*S. epidermidis*	15mm	11mm	7mm

Maximum ZOI 25mm was shown by methanolic extract of ajwain seeds against *Klebsiella pneumoniae* and the least ZOI was 7mm in *E. coli* and *S. epidermidis,* shown by aqueous extract of ajwain seeds (Table 3).

D. ZONE OF INHIBITION (ZOI) of *Piper nigrum* Seed (black pepper) Extract:

Methanolic Extract: In methanolic extract of black pepper seeds showed 16mm, 15mm, 13mm ZOI against *B. subtilis, S. aureus, S. epidermidis* (gram positive bacteria) respectively and 12mm, 10mm ZOI against *Klebsiella pneumoniae, E. coli* (gram negative bacteria) respectively.

Ethanolic Extract :14mm ZOI was showed by black pepper seeds extract against *S. aureus* and *Klebsiella pneumoniae* and 13mm, 12mm, 10mm ZOI was shown against *E. coli, S. epidermidis, B. subtilis* respectively.

Aqueous Extract: 6mm ZOI showed by aqueous extract of black pepper seeds against *E. coli* and *S. aureus*, 5mm ZOI was found in *S. epidermidis.* No antibacterial activity against other two bacteria.

Table 4. Anti-bacterial activity of *Piper nigrum* seed (black pepper) extracts – ZOI (mm)

BACTERIA		METHANOLIC EXTRACT 500(µg/disc)	ETHANOLIC EXTRACT 500(µg/disc)	AQUEOUS EXTRACT 500(µg/disc)
1.	*E. coli*	10mm	13mm	6mm
2.	*Klebsiella Pneumoniae*	16mm	10mm	NA
3.	*S. aureus*	15mm	14mm	6mm
4.	*B. subtilis*	16mm	10mm	NA
5.	*S. epidermidis*	13mm	12mm	5mm

Maximum ZOI was 16mm by methanolic extract of black pepper seeds against *B. subtilis* and ethanolic extract showed more anti-bacterial activity

against gram negative bacteria (14mm ZOI in *Klebsiella pneumoniae* and 13mm ZOI in *E.coli*) and against Gram positive methanolic extract showed more anti-bacterial activity as compared to ethanolic extract**.** The aqueous extract showed least anti-bacterial action with maximum ZOI 6mm in *E. coli* and *S. aureus* (Table 4).

Comparative analysis of ZOI between Methanolic, Ethanolic, Aqueous extract with Antibiotics:

EET = Ethanolic extract of *Trachyspermum ammi* seed

AEP = Aqueous extract of *Piper nigrum* seed

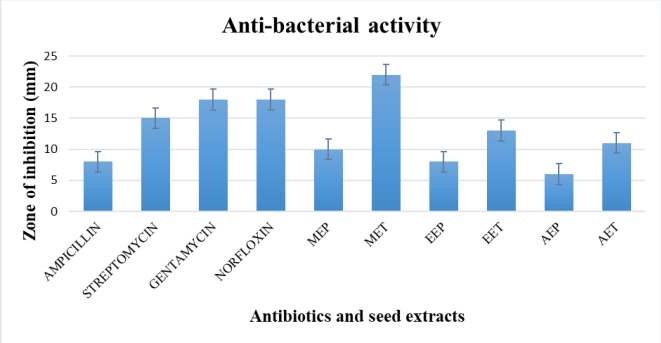
AET = Aqueous extract of *Trachyspermum ammi* seed

MEP = Methanolic extract of *Piper nigrum* seed

MET = Methanolic extract of *Trachyspermum ammi* seed

EEP = Ethanolic extract of *Piper nigrum* seed

1. *E. coli*



Graph 1. ZOI of Antibiotics, Ajwain and Black pepper in *E. coli.*

In *E.coli* the maximum ZOI of inhibition was showed by methanolic extract of *Trachyspermum ammi* seeds (MET) that was 22mm greater than reference antibiotics (Graph 1 and Fig. 1).

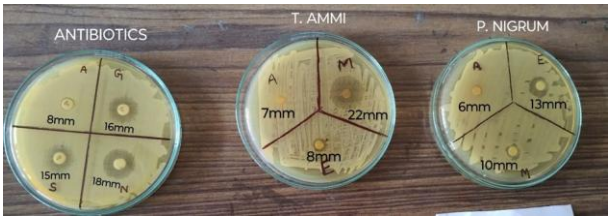
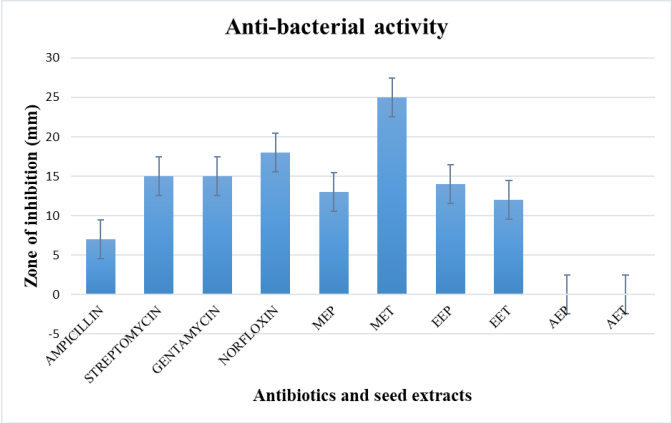


Fig. 1. ZOI of Antibiotics, Ajwain and Black pepper in *E. coli.*

2. *Klebsiella pneumonia*



Graph 2. ZOI of Antibiotics, Ajwain and Black pepper in *Klebsiella pneumonia*

The maximum zone of inhibition showed in *Klebsiella pneumoniae* by MET 25mm which is greater than ZOI Ampicillin, Streptomycin, Gentamicin, Norfloxacin 7mm, 15mm, 15mm, 18mm respectively (Graph 2 and Fig. 2).

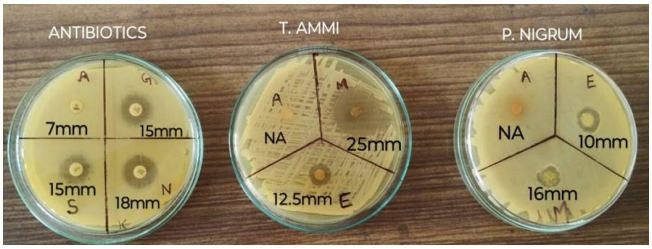
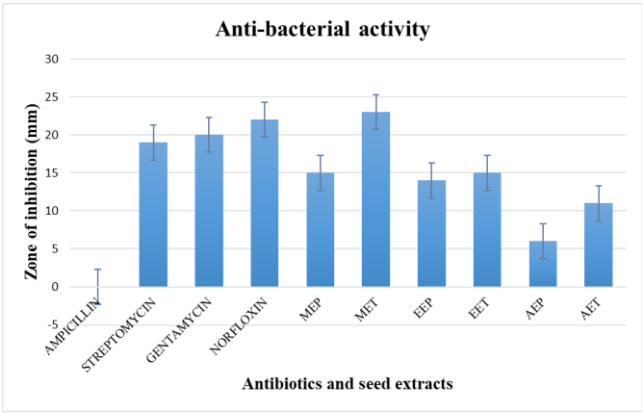


Fig. 2. ZOI of Antibiotics, Ajwain and Black pepper in *Klebsiella pneumonia*

3. *S. aureus*



Graph 3. ZOI of Antibiotics, Ajwain and Black pepper in *S. aureus.*

The maximum ZOI showed in *S. aureus* by MET that was 23mm greater than the ZOI showed by antibiotics Ampicillin, Streptomycin, Gentamicin and Norfloxacin. (Graph 3 and Fig. 3).

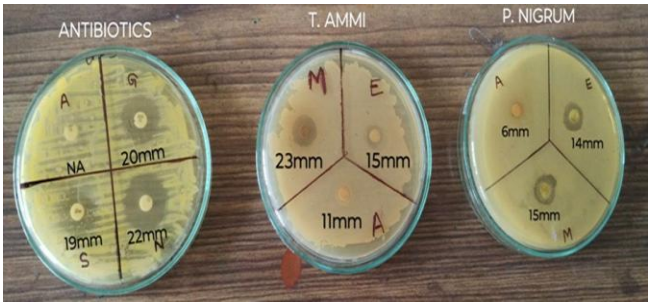
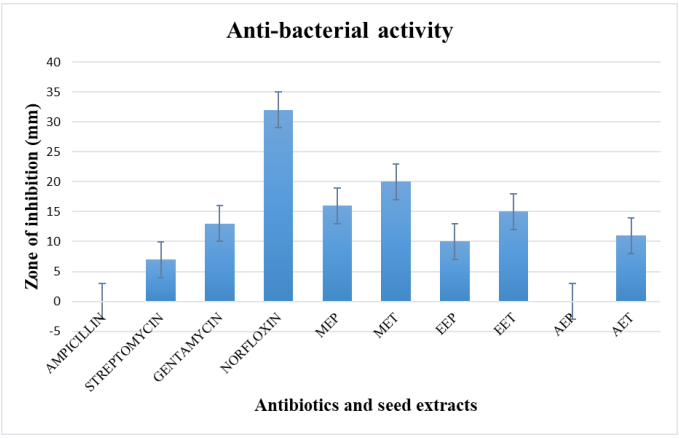


Fig. 3. ZOI of Antibiotics, Ajwain and Black pepper in *S. aureus.*

4. *Bacillus subtilis*



Graph 4. ZOI of Antibiotics, Ajwain and Black pepper in *Bacillus subtilis.*

The maximum ZOI showed in *B. subtilis* by MET that was 20mm greater than ZOI by Ampicillin, Gentamicin 7mm, 13mm respectively and nearest to the Norfloxacin antibiotic showed 32mm ZOI against this bacterium (Graph 4 and Fig. 4).

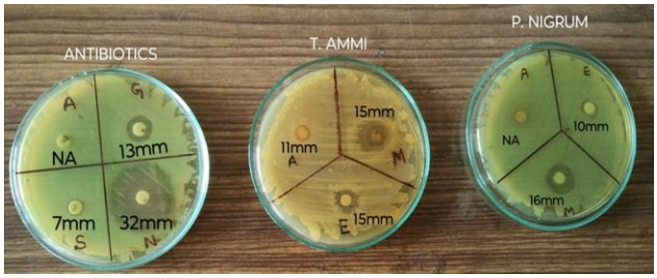
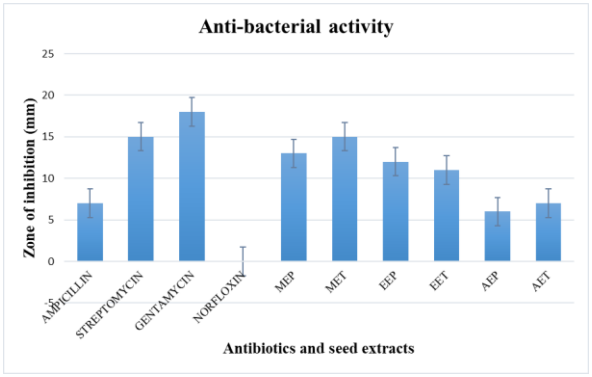


Fig. 4. ZOI of Antibiotics, Ajwain and Black pepper in *Bacillus subtilis.*

5. *S. epidermidis*



Graph 5. ZOI of Antibiotics, Ajwain and Black pepper in *S. epidermidis.*

The maximum ZOI showed in *S. epidermidis* by MET that was 15mm equal to streptomycin antibiotic which is used in control measure and nearest to the Gentamicin antibiotic that showed 18mm and greater than Ampicillin and Norfloxacin antibiotics 7mm, NA respectively. (Graph 5 and Fig. 5).

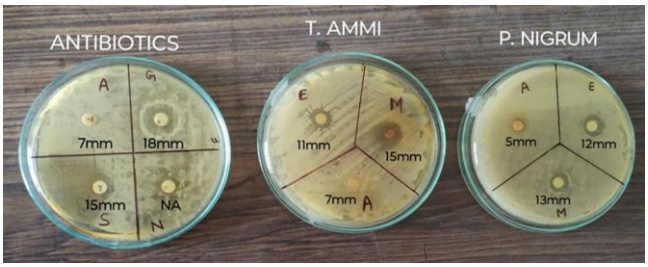


Fig. 5. ZOI of Antibiotics, Ajwain and Black pepper in *S. epidermidis.*

Discussion:

Antimicrobial resistance is a serious public health problem in which some bacteria that are causing serious diseases are becoming resistant to the commonly used antibiotics. It is estimated that 4·95 million deaths were associated with bacterial antibiotic resistance, including 1·27 million deaths attributable to bacterial AMR in 2019 (Murray *et.al.,*2022). Antimicrobial resistance (AMR) is critical to modern medicine and threatens the core of public health and can cause socioeconomic problem. Modern medicine depends on effective antimicrobial medicines, yet high rates of resistant infections across a broad range of microorganisms have been documented in all World Health Organization (WHO) regions, it is predicted that 10 million deaths will be attributed to AMR in 2050, if the silent pandemic is not addressed (O'Neill, 2016; Wang *et.al.,* 2021).

Plants represents an attractive source of antimicrobial agents with therapeutic potential as alternatives or potentiators of antibiotics (Khameneh *et.al.,* 2021). Current study revealed the antibacterial activity of *Trachyspermum ammi* and Piper nigrum methanolic, ethanolic and aqueous seed extract against numerous bacteria. The results showed that the ethanolic and methanolic extract of *Trachyspermum ammi* seed was effective against almost all bacteria and exhibit maximum inhibition against Klebsiella pneumoniae. However, the aqueous extract was less effective and exhibit maximum antibacterial activity against *S. aureus and B. subtilis*. On the other hand, methanolic and ethanolic extract of *Piper nigrum* seeds was effective against almost all bacteria and exhibit maximum inhibition against *Bacillus subtilis*. However, the aqueous extract of *Piper nigrum* showed less effect.

The observed activity showed that some bacteria strains have become drug resistant to antibiotics, as *S. aureus* and *B. subtilis* have become resistant to ampicillin antibiotics, other bacterial strain shows less than 8mm zone of inhibition and *S. epidermidis* gain drug resistance to norfloxacin antibiotic (Table 4).

The result of the present study is in contrast with those reported by Khan and Jameel (2018), their study revealed that the aqueous extract of ajwain possess no antibacterial property against *E.coli*, whereas in our study the aqueous solution possess 7mm ZOI.

These findings reveal that the natural compounds act as a potential remedy against various bacterial pathogens. Earlier research work on spices proved the presence of phytochemical composition, antioxidants, bioactive molecules etc that supports the effectiveness of these extracts of spices.

Similar results were obtained by the Nair *et al*., (2022). They assessed the antibacterial activity of *Trachyspermum ammi* and *Piper nigrum* extracts using different solvents and bacteria. The study revealed the inhibition of Gram positive and Gram negative bacteria is due to cell wall and cell membrane composition (Sharma *et al*., 2002).

Trachyspermum ammi has therapeutic use in traditional medicines and it's oil exhibited the strong antibacterial activity (Khan *et al*., 2018).

The phytochemical study of ajwain seeds showed the presence of saponins, tannins, flavone, mineral matters like nicotinic acid, thymol etc. which supports its antibacterial activity (Godavari *et al.,* 2021). Other researchers also showed the importance of ajwain oil in the antibacterial activity (Patil *et al*., 2016).

Piper nigrum also used for the treatment of various diseases (Sahrawat *et al.,* 2017). Its strong spicy taste comes from Piperine, which is a bioactive molecule (Reshmi and Justin, 2020). Pepper contains Glutathione peroxidase, Vitamin E, Terpinene, Piperamide, Pipene, which has diverse pharmacological activity (Aqil *et al.,* 2006). Piperine performs as a potent antibacterial activity (Hikal, 2018). Spices can be consistently used for food and medicinal purpose (Jiang, 2019).

Conclusion:

Spices have contributed a lot to the traditional medicine system. As the spices are less expensive and safer to use with negligible side effects, it is used in many home remedies to treat many diseases. The results showed that the presence of different natural antibacterial substances in methanol, ethanol and aqueous extract of *Trachyspermum ammi* and *Piper nigrum* had a great potential to inhibit the growth of various bacterial pathogens. The observed antibacterial activity of the extracts showed promising inhibitory action against both Gram negative and Gram positive pathogens. The antibacterial efficacy of the antibiotics was compared with these spices extracts and the extract showed their competitive antibacterial potency. It had also been observed that those bacterial strains which has gained resistance against the antibiotics showed some zone of inhibition in the ethanolic, methanolic and aqueous extract of the spices and inhibits their bacterial growth. This comparative study with antibiotic showed the efficacy of the spice extracts and it can be used as a potential alternative or supplementary treatments in the field of antibacterial therapeutics.

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