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Comparative Antimicrobial Activity of *Trachyspermum ammi* (Ajwain) and *Cinnamomum verum* (Cinnamon)

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Abstract: *The aim of this study was to compare the antimicrobial activity of Trachyspermum ammi and Cinnamomum verum against two different bacterial pathogens Escherichia coli and Staphylococcus aureus. To fulfill this, methanolic extracts were prepared from seeds of Trachyspermum ammi and bark of Cinnamomum verum. Antimicrobial activity was determined by disc diffusion method. Various concentrations of plant extracts such as 5 microliter, 6 microliter, 7 microliter and 8 microliter were tested against the bacterial species. Trachyspermum ammi showed very effective antimicrobial activity against E.coli and S. aureus at all the doses. Whereas Cinnamomum verum showed less antimicrobial activity in comparison to Trachyspermum ammi against both E.coli and S. aureus at all the doses.*

Keywords: *Trachyspermum ammi, Cinnamomum verum, Escherichia coli, Staphylococcus aureus, methanolic extract, Disc diffusion method.*

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Introduction:

Ajwain (*Trachyspermum ammi*) is a medicinal plant which is mainly grown in Iran, India, Pakistan and Egypt. Ajwain is generally grown in October–November and should be harvested in the months of May–June. In India it is cultivated in many states like Bihar, Maharastra, Uttar Pradesh and other states of India (Bashyal and Guha, 2018). Ajwain has been used as culinary spice all over the world and resembling thyme has traditional food flavoring and as a digestive stimulant or to treat disorders like of liver (Bashyal and Guha, 2018). It is also used in cough, dysphonia, nausea, vomiting, reflux and abdominal cramps (Zarshenas et al., 2013).

Cinnamomum verum or cinnamon is a plant species belonging to family Lauraceae (Shan et al., 2007). Cinnamon comes from Greek word 'Kinnamonum 'meaning sweet wood (Vinitha and Ballal, 2008). *C. verum* true cinnamon is extensively cultivated in Sri Lanka and Southern India. It is extensively used spice and is derived from inner bark of the tropical and evergreen cinnamon tree. The bark is collected and is allowed to dry, upon drying the bark rolls into tubular shape and is called Cinnamon stick or quill (Ziegenfuss et al., 2006). Cinnamon has been used in traditional medicine and as an spice in food preparation by the Egyptians and Chinese since long ago (Elshafie et al., 2012). The spice has antioxidant, antibacterial, antipyretic and anti-inflammatory properties playing major role in tissue healing and repair (Molania et al., 2012). The bark is used in tea and in herbal preparation for treating common cold, gastrointestinal and gynecological disorders (Hong et al., 2012). Cinnamon is also used to treat sore throats, cough, abdominal cramps, indigestion, spasms, diarrhoea and others (Vinitha and Ballal, 2008). Studies also suggest that Cinnamon delays food spoilage and shows antifungal properties (Elahi, 2012). *Escherichia coli* normally reside in the human and animal intestine. Usually the bacteria are harmless or can cause diarrhoea. But few strains can cause severe intestinal cramps and bloody diarrhoea. People or animals get exposed to the bacteria from contaminated food or water especially while consuming raw or undercooked vegetables (Barrett et al., 1994). *Staphylococcus aureus* is usually found in upper respiratory tract and on skin. It causes a wide variety of clinical diseases. The bacterium usually do not cause skin infection but enter internal tissue and blood stream causing variety of infections (Steven et al., 2015).

Herbs are natural products and their chemical composition varies looking on many factors and so vary from individual to individual (Fabio and Gori, 2007). Herbal medicine is also effective in curing infection such as *Staphylococcus* species that causes fever, cough, cold and skin disease (Saini and Porte, 2015). By using herbal medicines *E.coli,* the most common intestinal and non pathogenic organism, shows most antimicrobial effect against different herbs (Venugopal et. al., 2009). Herbal remedies are central to ayurveda and very widely used in daily purpose (Ptwardhan and Chopra, 2003) Many spices are used in treating diseases and improving general health (Ishani et. al., 2020)

Materials and Methods:

Materials: The seeds of *T. ammi* and bark of *C. verum* were purchased from local shops of Patna. The bacterial strains were collected from Central Research Laboratory (CRL), Patna Women's College. Chemicals used in the experiment were standard antibiotic discs [Gentamicin, Kanamycin and Penicillin], Muller Hinton Agar powder (Microgem), Luria broth powder (Himedia) and methanol (Finar chemicals). The research was carried out in CRL, Patna Women's College.

Plant extracts preparation: For extract preparation, the seeds of *T. ammi* and *C. verum* were grounded using mortar and pestle.100 gm each of powdered seed of *T. ammi* and bark of *C. verum* were soaked in 200 ml methanol in separate beakers. The beakers were covered with aluminum foil and kept aside for 24 hours for the components of the powdered seed and bark to get dissolved in methanol. After 24 hours the mixture was filtered with the help of muslin cloth. The filtrate collected was poured in clip boiling flask of rota evaporator. The extraction process was carried out for 15-20 min until the excess methanol evaporates and the extract becomes thick and sticky. The same extraction process was carried for both the plants extract preparation. Further, the extracts obtained

were collected in separate vials and both the extracts were stored in refrigerator for further use.

Bacterial broth preparation: Liquid broth medium was prepared by dissolving 1.3 gm of Luria bertani (LB) powder in 100 ml of distilled water. The solution was autoclaved at 121°C for 15-20 minutes. Two separate flasks each containing 50 ml of LB broth medium were used to grow bacterial culture. The collected strains of *E. coli and S. aureus* were used as inoculums. Bacteria were inoculated using inoculation loop in separate flasks. The bacterial transfers were carried out in laminar air flow bench. Both flasks were inoculated with different bacterial species. Both the liquid culture medium was kept in bacterial incubator at 37°C for 24 hours. The bacterial assay was carried out by disc diffusion method.

Agar medium preparation: Muller- Hinton Agar medium was prepared using MHA powder. 19 gm of MHA powder was dissolved in 500 ml of distilled water. The solution prepared was autoclaved at 121°C for 15-20 minutes. The medium was poured in petri plates in laminar air flow bench to avoid any form of contamination. The medium poured in petri plates allowed to cool down. The media plates were stored for further use.

Disc preparation: The discs were prepared by using Whattmann's filter paper no.4. The disc were soaked with different concentrations (5, 6, 7 and 8 microliter) of plant extracts and allowed to absorb the extract. The disc soaked in extracts were stored in separate Eppendorf for after use.

Disc diffusion method: The Disc diffusion assay for estimation of antimicrobial activity of plant extracts was performed with brief modifications as described earlier (Mandal et al., 2011). The whole process was carried out in sterilized and septic conditions. The agar media plates were inoculated with 150 microliter of *E.coli* culture and with 150 microliter of *S. aureus* separately, the culture was allowed to spread using glass spreader for bacterial culture to cover whole plate. Four discs of different dosage of Ajwain extract 5, 6, 7 and 8 l were taken and were placed in media plates containing *E. coli*. and other four discs of different dosage of Ajwain extract 5, 6, 7 and 8 microliter were placed in *S. aureus* culture plate separately. Similarly, four discs of different dosage of Cinnamon extract 5, 6, 7 and 8 l were taken and were placed in media plates containing *E. coli.* and other four discs of different dosage of Cinnamon extract 5, 6, 7 and 8 l were placed in *S. aureus* culture plate separately. All the culture plates were incubated at 37°C for 24 hours. In present study Kanamycin and Gentamicin were taken as positive control.

Results and Discussion:

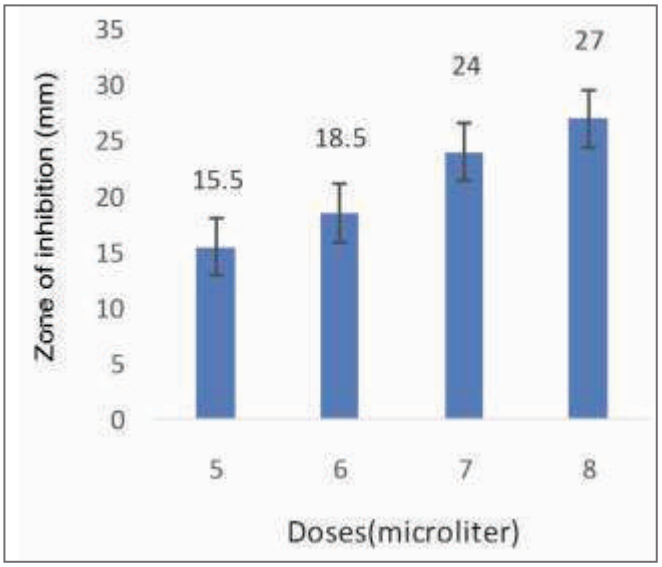

Fig. 1. Antimicrobial activity of *T. ammi* on *E.coli.*

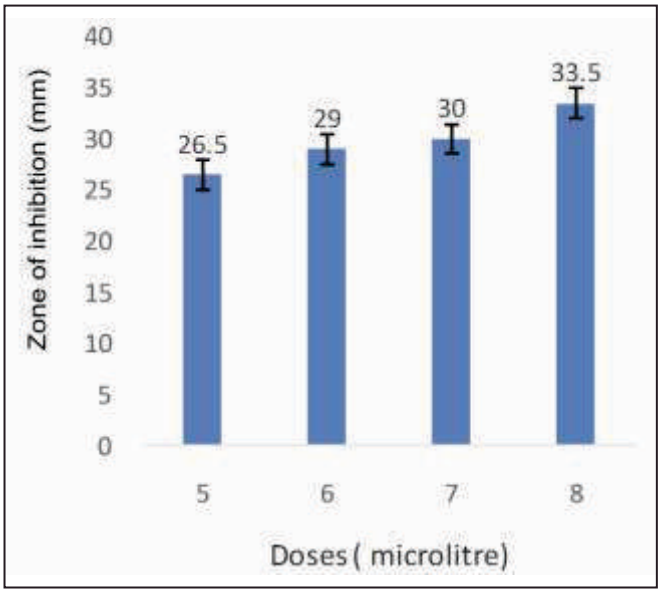

Fig. 2. Antimicrobial activity of *T. ammi aginst S.aureus*

The assay was prepared by disc diffusion method as defined in material and methods. In our study, methanolic seed extract of *Trachyspermum ammi* and methanolic bark extract of *Cinnamomum verum* were prepared to test their antimicrobial effect on bacterial pathogens *Escherichia coli* and *Staphylococcus aureus*.

Different doses of *T. ammi* extract 5, 6, 7 & 8 microliter were taken for results. The antimicrobial activity of methanolic seed extract of *T. ammi* (Fig. 1) was studied against bacterial pathogen *E. coli*. It was observed that the maximum zone of inhibition at concentrations 5, 6, 7 & 8 microliters were 15.5±0.7 mm, 18.5±0.7 mm, 24±2.8 mm and 27±1.4 mm respectively. Different doses of *T. ammi* extract 5, 6, 7 and 8 microliter were taken for results. The antimicrobial activity of methanolic seed extract of *T. ammi* (Fig. 2) was studied against bacterial pathogen *S.aureus*. It was observed that the mean diameter of inhibitory zones at doses 5, 6, 7 and 8 microliter were 26.5±2.1 mm, 29±2.8 mm, 30±2.8 mm and 33.5±0.7 mm respectively. This shows that antimicrobial activity of *T. ammi* on bacterial pathogens *E.coli* and *S. aureus* increases with increasing doses. The same was also observed by Godavari and Kumar (2021) and Rao and Gan (2014) on *T. ammi* and *C. verum* respectively on various bacterial strains.

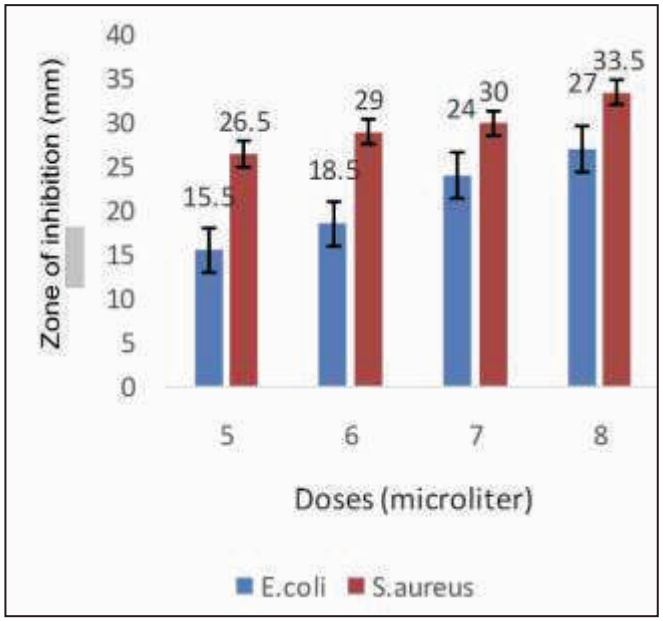

Fig. 3. Comparative graph of *T. ammi* on *E.coli* and *S.aureus*

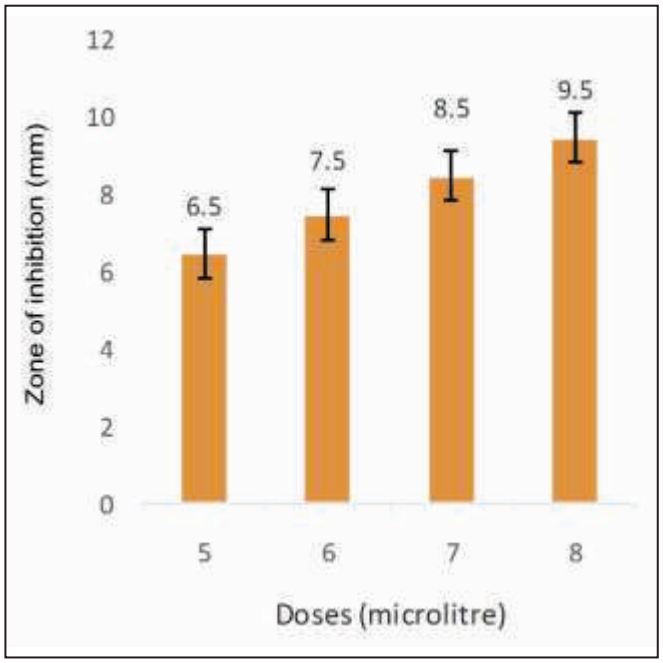

Fig. 4. Antibacterial activity of cinnamon *on E.coli*

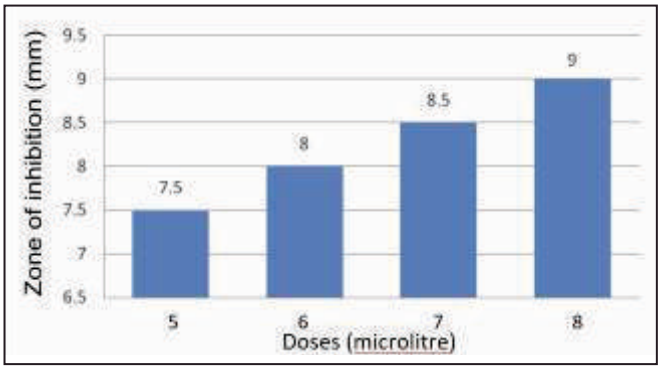

Fig. 5. Antibacterial activity of *C. verum* on *S.aureus*

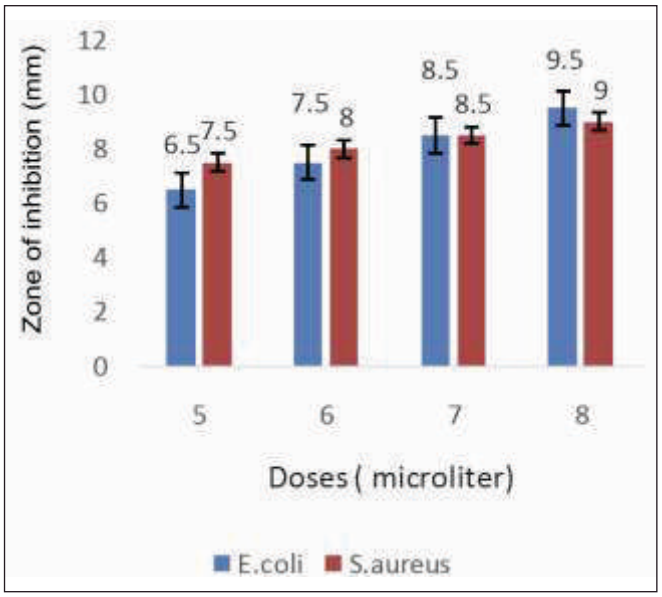

Fig. 6. Comparative graph of *C. verum* on *E.coli and S.aureus*

For comparative Ajwain extract antimicrobial study on *E. coli* and *S. aureus,* different doses of *T. ammi* extract 5, 6, 7 & 8 microliter were taken for results and observed on bacterial *E. Coli* and *S. aureus*. It was found that antimicrobial activity of *T. ammi* is more potent on pathogen *S. aureus* as compared to *E. coli* as higher zones of inhibition were observed (Fig. 3 Fig. 7A and B).

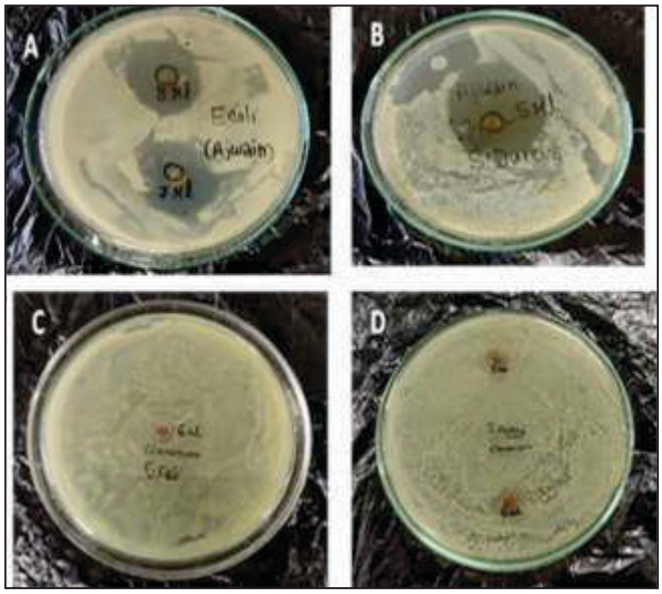

Fig. 7. Zone of inhibition of *T. ammi* on (A) E.coli (B) *S.aureus*. Zone of inhibition of Cinnamon against (C) *E. coli* (D) *S. aureus*

Different doses of *C. verum* extracts 5, 6, 7 & 8 microliter were taken for results. The antimicrobial activity of methanolic bark extract of *C. verum* was studied against bacterial pathogen *E. coli*. It was observed that the mean diameter of inhibitory zones at doses 5, 6, 7 and 8 microliter were 6.5±0.7 mm, 7.5±0.7 mm, 8.5±0.7 mm and 9.5±0.7 mm respectively (Fig. 4). The antimicrobial activity of methanolic bark extract of *C. verum* was also studied against bacterial pathogen S. aureus. It was observed that the mean diameter of inhibitory zones at doses 5, 6, 7 and 8 microliter were 7.5±0.7 mm, 8 mm, 8.5±0.7 mm and 9 mm respectively (Fig. 5). The result showed that antimicrobial activity of *C. verum* on bacterial pathogens *E.coli* and *S. aureus* increases with increasing doses.

For comparative cinnamon extract antimicrobial study on *E. coli* and *S. aureus,* different doses of *C. verum* extracts 5, 6, 7 & 8 microliter were taken for results and observed on bacterial *E. Coli* and *S. aureus*. It was observed that antimicrobial activity of *S. aureus* has similar effects

on pathogen *S. aureus* and *E. coli* as similar zones of inhibition can be observed (Fig. 6).

For comparative antimicrobial activity of Ajwain and Cinnamon on bacteria *E. coli* and *S. aureus*, different doses of *T. ammi* and *C. verum* extract 5, 6, 7 & 8 microliter were taken for results and observed on bacterial pathogen *E. coli*. The potential of antimicrobial activity of *T. ammi* methanolic seed extract and *C. verum* methanolic bark extract were compared. It was observed that *T. ammi* shows greater inhibitory zones as compared to that of *C. verum* at same doses i.e., 5, 6, 7 & 8 microliter.

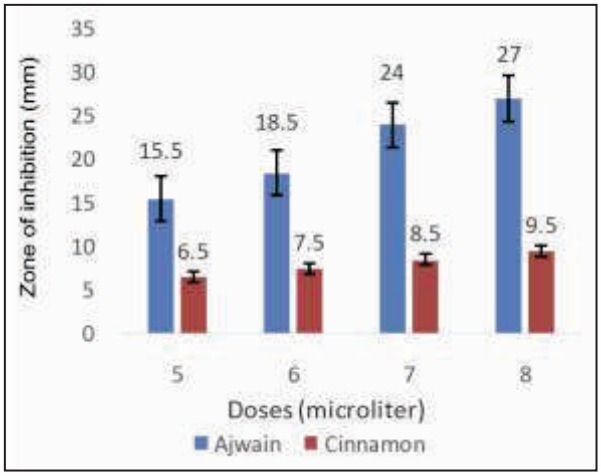

Fig. 8. Comparative graph of ajwain (*T. ammi)* and cinnamon (*C. verum*) on *E. coli*

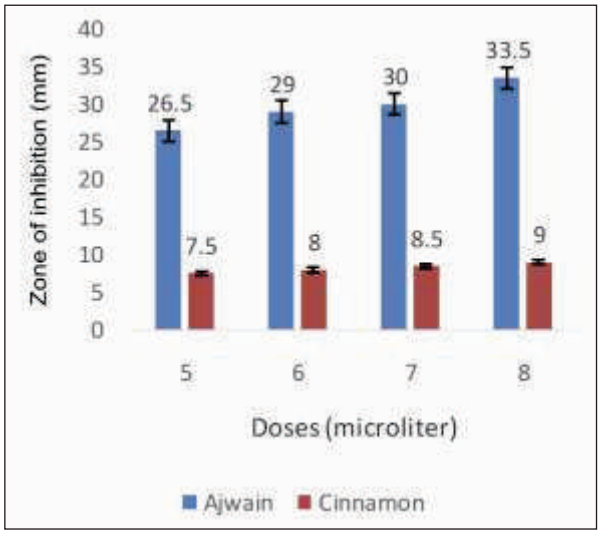

Fig. 9. Comparative graph of ajwain and cinnamon on *S. aureus*

For comparative antimicrobial activity of Ajwain and Cinnamon on bacteria *E. coli*, Different doses of *T. ammi* and *C. verum* extract – 5, 6, 7 & 8 microliter were taken for results and observed on bacterial pathogen *E. coli.* The potential of antimicrobial activity of *T. ammi* methanolic seed extract and *C. verum* methanolic bark extract were compared (Fig. 8). It was observed that *T. ammi* shows greater inhibitory zones as compared to that of *C. verum*.

For comparative antimicrobial activity of Ajwain and Cinnamon on bacteria *S. aureus*, Different doses of *T. ammi* and *C. verum* extract – 5, 6, 7 & 8 microliter were taken for results and observed on bacterial pathogen *S. aureus*. The potential of antimicrobial activity of *T. ammi* methanolic seed extract and *C. verum* methanolic bark extract were compared (Fig. 9). It was observed that *T. ammi* shows greater inhibitory zones as compared to that of *C. verum*.

Also, in Indian medicine Ajwain seeds are used as a household remedy against stomach disorder and asthma (Anonymous, 1995). Ajwain seeds contain fibers, carbohydrates, tannins, glycosides, moisture, protein, fat, saponins, flavone and mineral (Chauhan et. al., 2014). According to Ansari (1995) Ajwain seeds not only has antimicrobial properties but is also possess antifungal properties against several fungi. Cinnamaldehyde present in Cinnamon is an active agent to check the growth of both antibiotic resistant and antibiotic sensitive microbes (Ali et al., 2005).

Trachyspermum ammi and *Cinnamomum verum* are important medicinal plants, which have both medicinal and nutritional uses.

Conclusion:

Among both the plant extracts tested *T. ammi* showed much greater diameter for zone of inhibition against both the bacteria (*E. coli* and *S. aureus*) as

compared to the inhibitory effect of *C. verum* on the same bacteria. This shows that *T. ammi* is more effective than *C. verum.* Hence *T. ammi* can be used as a potent source of natural drug for antimicrobial sensitivity.

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