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Synergistic effect of Ajwain (Trachyspermum ammi) and Clove (Syzygium aromaticum) Aqueous extract on antibacterial activity

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Abstract

Ajwain (Trachyspermum ammi) is the important seed spice of the family Apiaceae. and is medicinally used as a diuretic, antivomiting, carminative, and anthelmintic agent. Cloves (Syzygium aromaticum) belong to the family Myrtaceae and are known to possess antimicrobial and antioxidant properties. In the present study, the antibacterial activity of the aqueous extracts of Ajwain (Trachyspermum ammi) and clove (Syzygium aromaticum) in combination has been evaluated against E. coli. In this study, we have determined that Trachyspermum ammi aqueous extract has resulted 45% and 60 % inhibition in the growth of E. coli at 60% and 90% w/v of the concentration, respectively. Moreover, in the case of Syzygium aromaticum, inhibition in the growth of E. coli observed was 35% and 45% respectively. However, in case of combination the inhibition observed was 70% and 85% in the growth of E. coli at the respective concentrations. In conclusion, the Trachyspermum ammi and Syzygium aromaticum aqueous extract show synergistic effect in the inhibition in the growth of E. coli as compared to alone.

Keywords:- Antibiotics; E. coli; Syzygium Aromaticum; Trachyspermum Ammi; Thymol; Carvacrol

1. Introduction

Plant-derived products have gained wide popularity among the scientific community to explore their various medical applications. Among several plant-derived products, spices have gained much interest to explore their efficacy as promising therapeutics and other medicinal benefits. According to literature, the spices are known to possess antianalgesic, antipyretic, and antiseptic properties (Coates et al. 2002; Braga et al. 2005). The spices are also found to be rich in antioxidants and possess antibiotic properties (Gilani et al. 2005). According to literature, spices generate reactive oxygen species, which leads to oxidative modification of DNA, proteins, and lipids in the affected cell, thereby being responsible for playing an important role in a wide range of common diseases like cancer, atherosclerosis, cardiovascular diseases, Alzheimer's, and other inflammatory responses (Neha et al. 2011; Bahareh et al. 2011).

Ajwain (carom) (wild parsley) (Trachyspermum ammi) is an aromatic compound belonging to the family of Apiaceae and is grown mostly in Iran, India, Pakistan, and Egypt (Oroojalian et al. 2010). According to literature, trachyspermum ammi is found to contain the phenols, mainly thymol and some carvacrol. The thymol and carvacrol present in trachyspermum ammi are mainly responsible for antiseptic and antitussive properties. Moreover, the antioxidant properties of trachyspermum ammi are due to the presence of thymol. Furthermore, trachyspermum ammi exhibits antiviral, anti-inflammatory, antimicrobial, antihypertensive, antispasmodic, and hepatoprotective properties (Brull and Coote (1999); Hussein et al. 2000; Aeschbach et al. 2004; Avagal and Rajeshwar (2022); Ozma et al. 2025; Wasim et al. 2024). Cloves (Syzygium aromaticum) are dried unopened floral buds of plant species. It belongs to the family Myrtaceae and is used in cooking as a spice because of its medicinal benefits and aroma. According to literature, cloves have been reported to be used for treatment of dyspepsia and gastric infection and also found to possess antimicrobial and antioxidant properties (Shyamala et al. 2003; Fu et al. 2007; Abdolhamid et al. 2011; Alanazi et al. 2022; Maggini et al. 2024). According to literature, antibiotics play the major role in fighting against bacterial and fungal infections (Thangam and Dhanajayan, 2003). However, plant-derived products that can serve as antibiotics against bacterial infections have more beneficial effects as compared to chemical

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compounds (Bonjar and Karimi, 2003). With the recent demand due to high rate of infection of pseudomonas aeruginosa, staphylococcus aureus, enterococcus feacalis and acinetobacter baumanni it has become important globally to rule over such incidence by exploring synergistic effects of muti plant derived products for better therapeutic potential (Coates et al. 2002; Akram et al. 2007).Thus, it becomes a global need to enhance the efficacy of available antimicrobial plant-derived agents or to explore new antimicrobial agents with strong inhibitory effects. In the present study, we have explored the synergetic effect of trachyspermum ammi and syzygium aromaticum aqueous extract at varied concentrations (5%, 10%, 15%, 30%, 60%, 90%) against E. coli.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant materials

The seeds of trachyspermum ammi and syzygium aromaticum used in this study were purchased from the local market of Mehsana, Gujarat, India. The seeds were identified by Department of Biotechnology, Ganpat University.

2.2 Preparation of extract

The trachyspermum ammi and syzygium aromaticum seeds were weighed and were then crushed. The distilled waters were added to the crushed trachyspermum ammi and syzygium aromaticum seeds, respectively, to attain varied concentrations (5%, 10%, 15%, 30%, 60%, 90% w/v.). The extract is then heated for 30 minutes at 60°C. After cooling, the extract was filtered through Whatman filter paper 1, and the extract collected was then preserved at room temperature for further analysis in an airtight container.

Fig. 1: Images of trachyspermum ammi and syzygium aromaticum Aqueous Extract (Left to Right A. Ajwain extract, B. Clove extract)

2.3 Bacterial test isolates

The standard strains of E. coli are collected from Department of Biotechnology, Ganpat University. The bacteria were cultured on nutrient agar medium and incubated at 37ºC for 24 hours.

2.4 Inhibitory effect of trachyspermum ammi Aqueous Extract and syzygium aromaticum aqueous extract as well as in combination against E.Coli

The inhibitory effects of trachyspermum ammi and syzygium aromaticum extract both in alone as well as in combination were determined by Agar dillution method against E.Coli.

2.5 Agar dilution method

The agar dilution method was performed to determine inhibitory effect of trachyspermum ammi (5%, 10%, 15%, 30%, 60%, 90% w/v.) and syzygium aromaticum (5%, 10%, 15%, 30%, 60%, 90% w/v.) aqueous extract both in alone as well as in combination with slight modification. In this method, sterilized petri plates were used to culture E. Coli. The method was conducted by pouring a 25ml nutrient agar on each plate which was then spread evenly. The E.Coli were culture at varied percentage of each extract (5%, 10%, 15%, 30%, 60%, 90% w/v.) and also in case of combination where both the extract were kept at 5%, 10%, 15%, 30%, 60%, 90% w/v. respectively. The inhibitory effect was compared with negative and positive control. For positive control 8µg/mL ampicillin was used and for negative control sterilized distilled water was used. The inhibitory effect was observed by incubating the culture at 37ºC for 24 hours. After incubation the percentage of resistance on E. coli culture plates in presence of trachyspermum ammi

and syzygium aromaticum aqueous extract at varied concentration both in case of alone as well as in combination were determined. For agar dilution, the suspensions were further diluted 1:1000 with distilled water. Bacterial suspensions were delivered to the surface using a multipoint inoculator.

3. RESULTS

The extract of trachyspermum ammi was light brown in color with a density of 1g/ml and the extract of clove was dark brown in color with density of 1g/ml as shown in fig 1. In this study, we have determined the synergetic effect of the trachyspermum ammi and syzygium aromaticum aqueous extract for their antibacterial activity against E. Coli.

In this study, Antibacterial activity was observed at varied concentration (5%, 10%, 15%, 30%, 60%, 90% w/v) of trachyspermum ammi and syzygium aromaticum aqueous extract. In case of combination significant inhibition was observed at all respective percentage as depicted in table 1 and fig. 2 and fig. 3.

Percentage of extract	Resistance of ajwain extract	Percentage of resistance of E.Coli ajwain Aqueous extract	Resistance of clove extract	Percentage of resistance of E. Coli in clove Aqueous extract	Resistance of mixture extract	Percentage of resistance of E.Coli in ajawain + Clove Aqueous extract
-ve control	++++++	0%	++++++	0%	++++++	0%
+ve control	-----	100%	-----	100%	-----	100%
5%	++++++	0%	++++++	~0%	+++++	~15%
10%	++++++	~8%	++++++	~5%	++++	~30%
15%	+++++	~16%	++++++	~10%	+++	~45%
30%	+++++	~20%	+++++	~15%	++	~60%
60%	+++	~45%	++++	~35%	++	~70%
90%	++	~50%	+++	~45%	+	~85%

Table 1: Percentage Inhibition of E coli in presence of Trachyspermum ammi and Syzygium aromaticum Aqueous Extract at varied Concentration (5%, 10%, 15%, 30%, 60%, 90% w/v.) both in case of alone as well as in combination

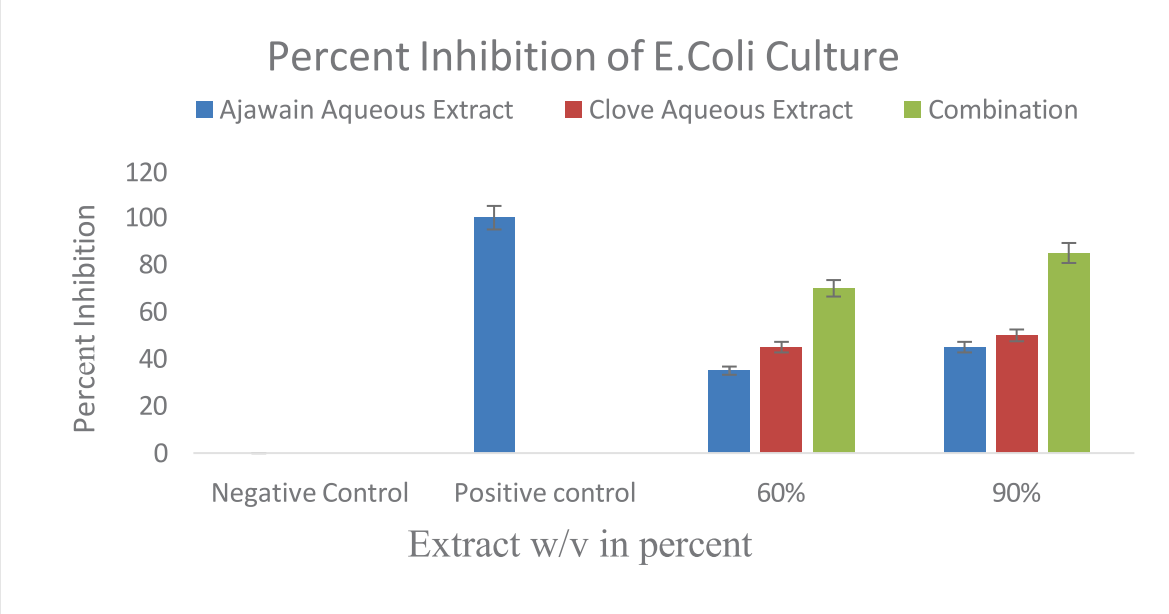

Fig. 2: Percentage Inhibition of E. coli in presence of Trachyspermum ammi and Syzygium aromaticum Aqueous Extract at 60% (w/v), 90% (w/v) concentration both in case of alone as well as in combination. Result is represented as ± SEM of three independent experiment. P value is calculated which was <0.05

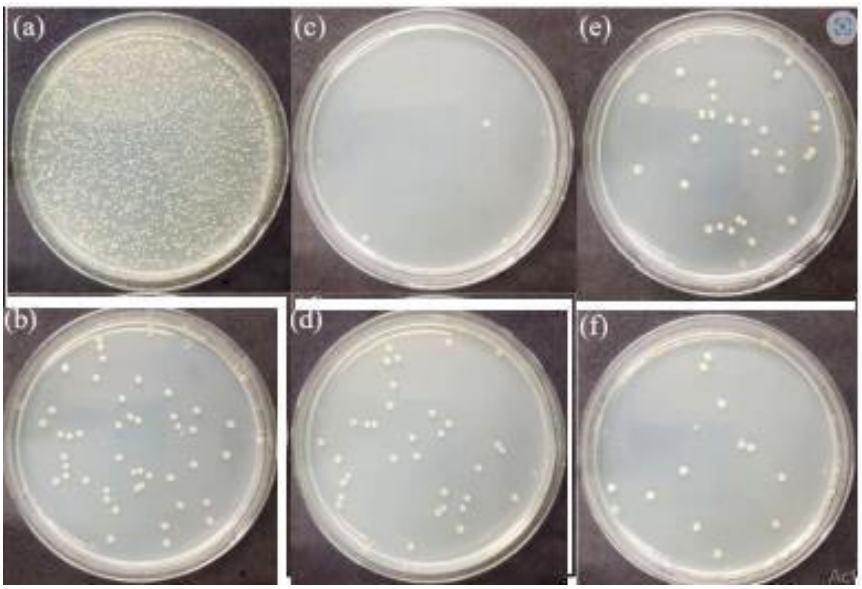

Fig. 3. Typical images of treatment of E. coli on nutrient agar plates (a) positive control (c) negative ciontrol (b) Syzygium aromaticum at 90% (w/v) (d) Trachyspermum ammi at 90 % (w/v), (e) combination at 60% (w/v), (f) combination at 90% (w/v). 1:1000 dilution of overnight culture of each isolate was prepared in sterile distilled water.

4. DISCUSSION

Trachyspermum ammi seeds have been reported to contains thymol, a phenol which is commonly used to treat digestive disorders. It is also found to possess antifungal and antibacterial properties. According to literature, trachyspermum ammi contained active enzymes which give patients relief during indigestion, bloating, and also helps to balance the release of acid in the small intestine during indigestion disorder. The trachyspermum ammi is commonly used to treat peptic ulcers and is also very effective in complications like sores in the esophagus or stomach, and in the intestine (Oroojalian et al. 2010).

According to reported studies the trachyspermum ammi, may also contained carvacrol and because of which it act as an inhibitor in the growth rate of bacteria and fungi. According to reported studies trachyspermum ammi was found to be potent antibacterial against salmonella and E.coli (Coates et al. 2002). The salmonella and E.coli are major bacteria that causes food poisoning and other digestive issues. Thus, Trachyspermum ammi can act strongly both as antifungal and antibacterial (Coates et al. 2002).

According to literature, syzygium aromaticum is used in as remedy in ayurveda against different health issues and diseases. Syzygium aromaticum is strongly used in ayurveda against diarrhea, hernia, and asthmatic issues (Fu et al. 2007). Syzygium aromaticum is also used to treat disorder related to small intestinal and also found effective in nausea, and vomiting. It is also used effectively in case of mouth and throat inflammation (Aeschbach et al. 2004).

The results show that when varied concentrations of trachyspermum ammi and syzygium aromaticum aqueous extract were used against the growth of E. coli in culture, they were found effective. Trachyspermum ammi aqueous extract inhibits 45% to 50% of the growth of E. coli at 60% and 90% of the extract. However, Syzygium aromaticum aqueous extract inhibits 35% and 45% of the bacteria growth at 60% and 90% w/v of the extract. Moreover, in the case of combination, the inhibition effects observed against the growth of E. coli are significant, up to 70% and 85% at 60% and 90% w/v of the extracts, respectively. In support of our findings, similar studies were reported on different gram-negative strains. (Alam et al. 2022; Belzawar 2016)

The antibacterial activity of the extract was increased with an increase in the percentage concentration of the extract. When we increased the percentage concentration of extract, the inhibition in antibacterial activity was also found to be increased. The study concludes that the combination of trachyspermum ammi and syzygium aromaticum aqueous extract significantly increased their antibacterial activity. This shows that in the case of the combination of trachyspermum ammi and syzygium aromaticum, possess a synergistic effect against the growth of E. coli.

CONCLUSION

In conclusion, trachyspermum ammi and syzygium aromaticum aqueous extract, when used at varied concentrations against the growth of E. coli, have shown an increase in inhibition in the growth rate with an increase in the percentage of the extract. However, when both extracts were used in combination, the inhibition in the growth of E. coli was found to be increased significantly. Thus, we can suggest that trachyspermum ammi and syzygium aromaticum aqueous extract in case of combination shows synergistic effects. Subsequently, both in case of combination are found to be more effective

against the inhibition in the growth of E. coli and can be used as potent antibacterial compounds. We can conclude based on our study that trachyspermum ammi and syzygium aromaticum extract, in case of combination, even in crude form, possess strong antibacterial activity.

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