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Combating E.Coli Resistance With Essential Oils: A Study On The Efficacy Of Ajwain And Clove Oil

Ms. Tanaya Jaipal Bhowate
Student at Pravara College of Pharmacy (For Women's), Chincholi, Nashik, Maharashtra

Mr.Vikas Kunde
Assistant professor at Pravara College of Pharmacy (For Women's), Chincholi, Nashik, Maharashtra

Abstract:

With the rise in antibiotic resistance, essential oils—known for their antibacterial qualities—are becoming more and more popular as natural substitutes for traditional antibiotics. The purpose of this study was to evaluate the antibacterial activity of two essential oils—clove (Syzygium aromaticum) and ajwain (Carum copticum)—against a resistant strain of Escherichia coli. The zone of inhibition and colony counting were the two techniques used in the study. Each essential oil was evaluated at varying concentrations (25µl, 50µl, 75µl, and 100µl) while the bacteria were cultured on nutrient agar and nutrient broth. The typical reference antibiotic that was used was amoxicillin. Clove oil showed a bigger inhibition zone than amoxicillin and ajwain oil, with diameters up to 26 mm, according to the zone of inhibition technique results. Further confirming clove oil's greater antibacterial activity, the colony counting method showed a higher inhibition percentage for clove oil (88.3%) than for ajwain oil (75%). The main reason clove oil works so well is because of eugenol, a phenolic component that breaks down bacterial cell walls and prevents vital biological processes. Thymol-containing ajwain oil also demonstrated strong antimicrobial properties. The potential application of essential oils, especially clove oil, as strong antibacterial agents against resistant E. coli is highlighted by this study.These results imply that essential oils may be crucial in creating alternative treatment approaches, which is noteworthy given the rise in antibiotic resistance.

Keywords: Ajwain oil, Clove Oil, Amoxicillin, Antibacteria

INTRODUCTION

Essential oils, which are concentrated plant extracts, are gaining popularity due to their potent antibacterial enzyme activity and ability to stop the growth and reproduction of microorganisms. to counteract the characteristics of antibiotic resistance. They offer a natural alternative to synthetic agents in a number of industries, such as food preservation, medicine, and agriculture. By pressing or distilling plants, essential oils—fragrant, aromatic liquids—are extracted while maintaining the plant's flavor and scent. (8) Essential oils are being researched as potential alternatives to manufactured antibacterial agents since it is thought that they exert an antibacterial impact by breaking down microbial cell membranes. (9)

Antibacterial properties, which are essential in scientific and industrial fields like medicine, agriculture, and cosmetics, are present in many essential oils. Of the 250 essential oils that are marketed commercially, about 12 have potent antibacterial qualities. (10) Given the increasing resilience of harmful microorganisms, essential oils may eventually take the place of synthetic materials. Essential oils, particularly those with hydrophobic properties, have the ability to permeate bacterial cell walls, causing damage and ultimately cell death. Ascarvacrol, eugenol, and cinnamonaldehyde are among the compounds in essential oils that can inhibit the enzymes responsible for ATP synthesis, a crucial stage in bacterial metabolism.

AIM AND OBJECTIVE

AIM: To compare the different concentration so essential oils against resistant microbe E.coli.

OBJECTIVE: Compare the active concentration so Essential oils against a resistant microbe, that is E.coli to draw out a direct comparison in terms of efficacy.

MATERIALS AND METHODOLOGY

Nutrient agar

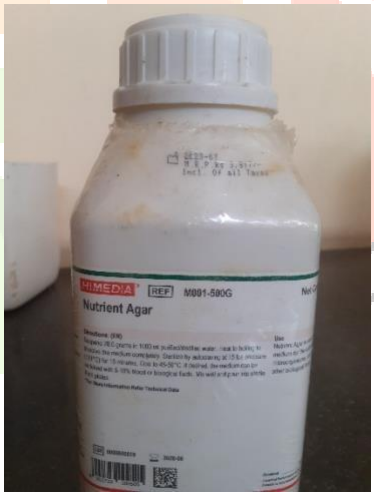


Fig1:Nutrient Agar

A general-purpose nutrient medium, nutrient agar is used to cultivate microorganisms that promote the growth of a variety of non-fastidious organisms. The culture of bacteria and the counting of organisms in water, sewage, feces, and other materials are two uses for nutrient agar. Because it includes numerous nutrients required for bacterial development and can cultivate a wide range of bacteria and fungi, nutrient agar is widely used. A wide range of bacteria that typically don't need particular nutrients or supplements can grow on nutritional agar because it is created with different nutrients. Agar, beef extract, and peptone are the main ingredients of the medium. These nutrients are supplemented with a few vitamins and trace substances that are essential for bacterial growth. Agar is a flexible medium for growing a wide variety of microorganisms. It is ideal for routinely cultivating and counting non-fastidious microorganisms due to its simplicity**.**

Nutrient broth

A versatile liquid medium, nutrient broth serves as the basis for numerous microbiological procedures and is used to cultivate a broad range of bacteria, particularly those with low nutritional needs. Peptone and yeast extract, which supply vital nutrients such nitrogen molecules, amino acids, and vitamins for microbial growth, are commonly found in nutrition broth. In microbiology, nutrient broth (NB) is a versatile liquid medium that is frequently used to cultivate a range of bacteria, including Escherichia coli (E.coli).In order to provide vital nutrients including amino acids, peptides, carbohydrates, vitamins, and minerals required for bacterial development, its composition usually consists of peptone, yeast extract, sodium chloride, and occasionally beef extract. Research has shown that E.Coli can continue to grow in nutrient broth for along time. For example, studies show that E. coli grows net in NB and other medium such as Luria-Bertani (LB) and Brain Heart Infusion (BHI) for up to four weeks. In microbiological research, nutrient broth is a useful medium for growing and maintaining Escherichia coli because it provides the vital nutrients needed for the organism's growth and survival.

E coli culture

The multi purpose bacteria Escherichia coli, or E.coli, is frequently found in both human and animal intestines. Some strains can cause serious infections, especially when they develop antibiotic resistance, although the majority are harmless. E.coli is capable of producing β-lactamases, which are enzymes that degrade β-lactam antibiotics, such as cephalosporins and penicillins, making them ineffective. By means of conjugation, transformation,or transduction, E.coli can obtain resistance genes from other bacteria. This makes it easier for resistance characteristics to proliferate among various bacterial populations. In both clinical and environmental contexts, E. Coli is frequently employed as an indicator bacterium to monitor changes in antibiotic resistance.

Autoclaving of agar

Fig2: Autoclaving

An autoclave is a device that kills any potential microbes by subjecting objects to high-pressure saturated steam at high temperatures. Use heat-protective gloves when putting or taking good sout of the autoclave because it can reach high temperatures and pressures. For agar media to be free of contaminating microorganisms, autoclaving is an essential step in the preparation process. Growing pure cultures of bacteria, fungus, or other microorganisms without interference from undesirable impurities requires this sterilizing procedure. The agar medium is heated to 121°C (250°F) for 15 to 20 minutes at 15 pressure as part of the normal procedure.

Incubation of the bacterial culture

Fig3:Test tubes with e coli culture

An E. Coli bacterial culture should be incubated in nutrient broth, the sterile broth containing the bacteria, for 12 to 18 hours at 37°C with aeration(shaking),and growth should be monitored added a tiny quantity of E.Coli culture to the sterile nutritional broth (for example, from a frozen stock or a streak plate).

Preparation of agar plates

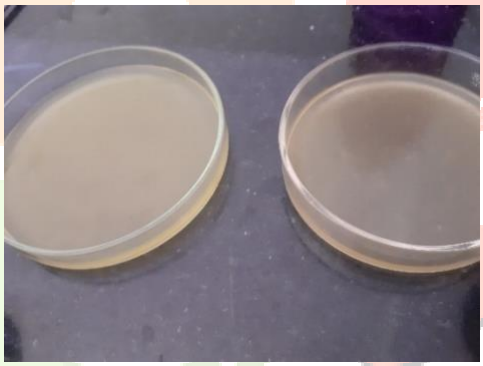


Fig4:Agar plates

It consists of the subsequent steps:

1. Agar Medium Preparation In distilled water, dissolve the recommended quantity of agar powder (e.g., 1.5% agar solution), stirring and boiling to ensure full dissolution. Filter the agar medium or autoclave it for 15 minutes at 121°C to sterilize it.
2. Sterilize Petri dishes by pouring and solidifying them: Make sure the petri dishes are sterile by using pre-sterilized ones or autoclaving them. Pour Agar: In order to prevent bubbles and ensure even distribution, carefully pour the melted agar into the sterilized petri dishes. Solidification: At room temperature, let the agar solidify.
3. Utilization and storage: Until they are ready to be used, keep the agar plates in a cold, dark location. Use: Let the plates come to room temperature before using them, then incubate them at the right temperature for the microorganisms being cultivated.

Incubation of agarplates

E. Coli cultures are cultured on agar plates. To give the bacteria time to develop and form colonies, place them upside down at 37°C for 14–18 hours (overnight).The bacteria can grow and create visible colonies on the plates if they are incubated for the entire night, usually 14–18 hours.

Tests included for comparative study:

Zone of inhibition: The zone of inhibition of different concentrations of ajwain oil and clove oil were compared with standard amoxicillin. It was found Clove oil have more potential antibacterial effects as compared to that of Ajwain oil. The results are shown in table 1,2 and 3

Table1: Different concentrations of amoxicillin in stock solution of 1mg/ml

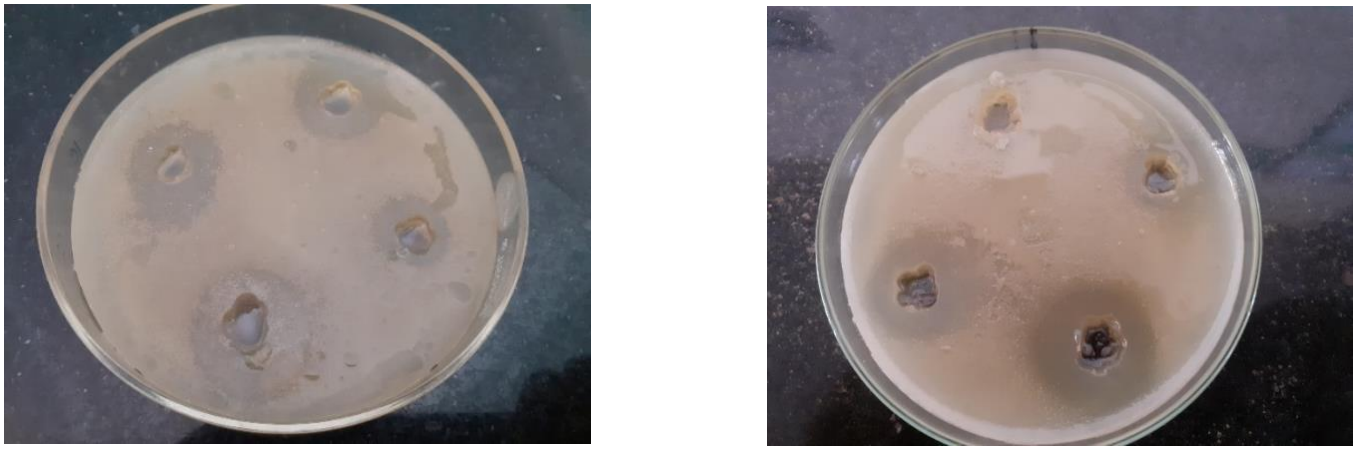
Concentrations (microlitre)	Zones	Diametre (mm)
25	A	21
50	B	22
75	C	22
100	D	23.5

Table2:Clove oil at different concentrations

Concentrations (microlitre)	Zones	Diametre (mm)
25	A	23
50	B	24
75	C	24
100	D	26

Table3:Ajwain oil at different concentrations

Concentrations (microlitre)	Zones	Diametre (mm)
25	A	20
50	B	20
75	C	22
100	D	23



(a)Clove Oil (b) Ajwain Oil

Fig5: Zone of Inhibition

Colony counting method: Colony counting method is performed in the microbiology to estimate the number of viable microorganisms in a sample by counting the colonies that grow on a solid medium after incubation. The colony counting method was performed on the different concentrations i.e. 25µl, 50µl, 75µl, 100µl of ajwain oil and clove oil using colony counter. The data was expressed in colony forming unit (cfu) and in that it was found that clove oil have greater inhibition percentage than that of ajwain oil.

Table4: Colony counting method for ajwain oil

CONDITIONS	PLATE1 (Cfu)	PLATE2 (Cfu)	PLATE3 (Cfu)	PLATE4 (Cfu)
CONTROL	258	290	314	283
TREATED WITHAJWAIN OIL	25µl	50µl	75µl	100µl
	190 (Cfu)	35 (Cfu)	28 (Cfu)	23 (Cfu)

Table5: Colony counting method for Clove oil

CONDITIONS	PLATE 1 (Cfu)	PLATE 2 (Cfu)	PLATE 3 (Cfu)	PLATE 4 (Cfu)
CONTROL	289	297	318	310
TREATEDWITH CLOVE OIL	25µl	50µl	75µl	100µl
	103 (Cfu)	17 (Cfu)	12 (Cfu)	10 (Cfu)

(a) Treatment with essential oil (b) Control e.coli culture

Fig6:Colony counting method

Formula

In microbiology, percentage inhibition refers to the extent to which a substance (like an antibiotic, plant extract, or chemical compound) reduces the growth or activity of a microorganism, usually compared to a control sample without the substance.

%Inhibition=Control-Treatment/Controlx100 where,
Control=growth observed without the inhibitory agent Treatment= growth observed with the inhibitory agent

RESULTS AND DISCUSSION

RESULT:

1. **Zone of Inhibition:** The zone of inhibition of different concentrations of ajwain oil and clove oil were compared with the standard drug amoxicillin. Later it was found that on the basis of the results that clove oil is more potent than that of ajwain oil.

2. **Colony Counting Method:** The colony counting method was performed on the different concentrations of ajwain oil and clove oil. The data was expressed in the form of cfu that is Colony forming unit. And it was found that in the presence of clove oil there is more antibacterial activity than that of the ajwain oil. The inhibition percentage was calculated on the basis of the total viable count of colony counting.

%Inhibition of Clove oil=88.3%
%Inhibition of Ajwain oil=75%

DISCUSSION:

The present study investigates the comparative effects of ajwain oil and clove oil against inhibition and antibacterial effects on E.coli. It was found that clove oil exhibits greater effects against equally as compared to ajwain oil. However both have valuable roll and significance in controlling the growth of bacteria as compared to the standard amoxicillin, as e coli is resistant to amoxicillin. Clove oil's zone of inhibition is greater than that of the standard used as well as ajwain oil.Due to the presence of eugenol in clove oil, that is a phenolic compound, it disrupts the bacterial cell membrane and inhibits the biofilm formation. However ajwain oil have also controlled the growth of bacteria due to the presence of bacteriostatic and bactericidal action towards e coli.

CONCLUSION

This study demonstrates the antibacterial potential of essential oils, specifically ajwain oil and clove oil, against resistant strains of *Escherichia coli*. Both oils exhibited significant antibacterial activity, but clove oil proved to be more effective, as shown by a larger zone of inhibition and higher percentage of growth inhibition(88.3%) compared to ajwain oil(75%).The greater efficacy of clove oil is attributed to its high content of eugenol, a potent phenolic compound known to disrupt bacterial membranes and inhibit critical cellular functions. While ajwain oil also showed antibacterial effects due to the presence of thymol and other active components, its impact was comparatively lower. Overall, the findings suggest that clove oil has promising potential as a natural antibacterial agent, particularly against antibiotic-resistant strains of *E. coli*, and could serve as a viable alternative or complement to conventional antibiotics in controlling microbial infections

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