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RESEARCH ARTICLE

Antibacterial activity and phytochemical analysis of natural herbs

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Abstract: In recent years, researchers from all around the world have come to the conclusion that any antimicrobial agent has a limited shelf life since microbes are increasingly developing resistance to it. In order to discover new alternative sources of antimicrobial agents, particularly from plants, several investigations have been carried out. Using chemical preservatives to prevent food deterioration and food poisoning pathogens has drawbacks, including risks to human health from chemical applications, chemical residues in food and feed chains, and the development of microbial resistance to the chemicals utilized. Finding alternative preservatives that may be effective, safe, and natural is becoming more important as a result of these worries. Plant extracts have been used to preserve food and prevent food-borne illnesses.Therefore,this study was conducted to determine the phytochemical analysis and antibacterial properties of *Mentha piperita*(mint),*Trachyspermum ammi*(Ajwain) and *Ocimum tenuiflorum*(tulsi) against *Escherichia coli* and *Salmonella typhi*. The methanolic extraction of plant leaves showed the presence of phenols and tannins, flavonoids, carbohydrates, glycosides and alkaloids. Higher antibacterial activity was observed with mint against *Salmonella typhi* and *Escherichia coli* with 14 mm and 16 mm zone of inhibition respectively followed by tulsi against *Salmonella typhi* and *Escherichia coli* with 11 mm and 14 mm respectively. Ajwain was not effective against both bacteria. Agar cup plate method was used to evaluate antibacterial effect of mint,ajwain and tulsi.

Keywords: Ajwain, Antibacterial activity, Bacteria, Mint, Tulsi

Introduction

Herbal plants have been used since the beginning of human civilization for the treatment of many illnesses. Many of these herbs, because of their pharmaceutical properties, became current drug candidates (Roessner *et al.* 2012). Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine (Bargali *et al.* 2021, Cutler and Cutler *et al.* 1999).Phytochemicals from plants have shown great promise in the treatment of intractable infectious human diseases including viral infections (Cowan *et al.* 1999). The aqueous extracts of *Camelia sinensis* and *Trachyspermum ammi* were found to be effective against *Salmonella* isolated from curry samples (Gunasegaran *et al.* 2011). Methanolic leaf extract of *Coleus amboinicus* leaves showed remarkable antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) (Sahgal *et al.* 2009). *Mentha piperita* (Lamiaceae), the peppermint (mint) plant is an aromatic perennial herb, cultivated in most part of the world, has traditionally been used in folk medicine. Leaves of mint plant are frequently used in herbal tea and for culinary purpose to add flavour and aroma. The distinctive smell and flavour, a characteristic feature of *Mentha spp*. is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain (Pramila *et al.* 2012). The mint plants are rich sources of iron and magnesium, which play important role in human nutrition (Arzani *et al.* 2007). A large volume of literature is available on the medicinal properties of essential oils present in *Mentha spp.* (Rasooli *et al.* 2008).

Ocimum tenuiflorum, also known as

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Ocimum sanctum, Tulsi, or Holy Basil from the family Lamiaceae has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities (Kumar *et al.* 2010). It has been one of the most valued and holistic herbs used over years in traditional medicine in India and almost every part of the plant has been found to possess therapeutic properties (Kumar *et al.* 2010). Leaves of *O. sanctum* have been shown to possess hypoglycemic effects in experimental animals (Muralikrishnan *et al.* 2012). *Trachyspermum ammi* (*T. ammi*), is another highly important medicinal plant. Fruits of *T. ammi* possess antiseptic, antifungal/antibacterial, and anthelmintic properties (Dubey *et al.* 2015). The seeds of this plant species contain 2 - 4.4% brown-colored oil known as ajwain oil. The main component of this oil is thymol, which is used for the treatment of gastrointestinal ailments, lack of appetite, and bronchial problems (Kowalczyk *et al.* 2020). The oil exhibits fungicidal, antimicrobial, and anti-aggregatory activities. In addition, the fruit has antispasmodic and carminative activities and is used traditionally as a remedial agent for many gastrointestinal and respiratory disorders. Most of the food poisoning diseases have been associated with bacterial contaminations particularly members of Gram negative bacteria like *E. coli* and *S. typhi*. Therefore, the present study aimed to evaluate antibacterial activity of these plant extract against *E. coli* and *S. typhi*.

Keywords: Ajwain, Antimicrobial activity, Bacteria , Mint, Tulsi

Material and Method

Bacterial strains

Two pathogenic bacteria were used in this study: *Salmonella typhimurium* and *Escherichia coli.*

Plant Material

Mentha piperita (mint), *Trachyspermum ammi* (Ajwain) and *Ocimum tenuiflorum* (tulsi) leaves obtained from the local nursery in Adalaj, Gujarat were used in this experiment. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of Plant materials

90 gram of dried leaves were crushed in powder. 50 grams of powdered Mentha piperita leaves were then extracted using the Soxhlet technique and methanol. As a solvent, 250 ml of methanol was utilised. A heating mantle was used to continually heat the apparatus. An extraction thimble was used to keep the sample, but the thimble was subsequently submerged in a boiling solvent. The Soxhlet extraction solvent, was put through several cycles over the course of about 6-7 hours, was collected, and the resulting solvent was concentrated by a rotary evaporator under the reduced pressure while maintaining a temperature below the boiling point of the methanol that was used as the extraction solvent. The extracts were kept at a very low temperature and stored in small vial tubes.

Identification of bioactive compunds in plants

This method involves the selective and successive extraction of the plant phytochemical. Various specialised reagents were used to analyse the primary group of natural compounds found in the plant extract. Standard procedures were used in chemical testing to discover bioactive substances with pharmacological significance. Phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, phenols, etc were determined qualitatively as following:

Phenol and tannins

2 drops of crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A black coloration indicates the presence of phenols and tannins.

Glycosides (Keller Kilianin Test)

2ml of extract was treated with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1ml of concentrated sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Flavonoids (Shinoda's test)

The extract was mixed with 2-3 magnesium ribbon fragments and 4 drops of conc. HCl was added. Red

coloration indicates the presence of flavonoids in the extract.

Reducing sugar

1ml of each extract was added to 1ml of Benedict's reagent. The mixture was heated on a boiling water bath for 2 minutes solution appeared green showing the presence of reducing sugar.

Alkaloids

Crude extract was mixed with 2ml of 2% HCl. Then, the mixture was heated on a boiling water bath, cooled, filtered and divided into two tubes.

Mayer's Reagent: To the solution of first tube, few drop of Mayer's reagent was added. Appearance of yellow precipitation or presence of turbidity indicates the presence of alkaloids.

Dragendorff's Reagent: To the solution of second tube, 3 drops of Dragendorff's reagent was added. Appearance of white precipitate indicates the presence of alkaloids.

Agar cup plate method for antibacterial activity

10% (w/v) methanolic extract and 20% methanolic extract of all three plant leaves were prepared. A 16 h culture was diluted with a sterile physiological saline solution [PS; 0.85% (w/v) sodium chloride with reference to the 0.5 McFarland standards to achieve an inoculum size of approximately 10^6 colony forming units/ml. Wells were made in nutrient agar plate using cork borer of approximately 6 - 8 mm and fifty micro-liters of the working suspension/solution of different medicinal plant extract and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Two cylindrical cavities were made in medium with the help of sterile borer. In one cavity 50µl of 10% **10ml** methanolic extract of medicinal plant was added while in other **20ml** 20% methanolic extract of medicinal plant was added. Plates were left for some time till the extract diffused in the medium with the lid closed and incubated at 37°C for 24 h. After overnight incubation the plates were observed for the zone of inhibition (ZI) and the diameter of the inhibition zone were measured using scale and mean were recorded. For negative control fifty micro-liters of water, 10% methanol and 20% methanol were transferred in three wells of another petriplates containing test organisms. The petridish was specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium is in uniform thickness.

Results

Phytochemical analysis

The different phytochemical constituents present in the extract were identified by the color reaction with different reagents. The main objective of phytochemical screening was to identify the different groups of chemical constitutents present in the plant extract. Results of the phytochemical screening are shown in table 1.

A preliminary study has reported that the leaf extract of plants contains large number of bioactive secondary molecules like phenols, alkaloids, tannins, glycosides, carbohydrates, flavonoids (Table 1). The presence of these components in this species is an indication that it may have some medicinal potential.

Antibacterial activity

Mint and Tulsi were found to be effective in inhibiting the growth of both *Escherichia coli* and *Salmonella typhi* while ajwain was not effective against both bacteria as shown in table 2, figure 1 and 2. Negative control containing water, 10% methanol and 20% methanol without leaves of plants showed no antibacterial activity against both bacteria as shown in figure 3. 20% methanolic plant

Table 1: Phytochemical screening of methanolic extract of tulsi, mint and ajwain leaves

Sr. No.	Phytochemicals	Extract of tulsi leaves	Extract of mint leaves	Extract of ajwain leaves
1	Phenol	+	+	+
2	Tannins	+	+	+
3	Glycosides	+	+	-
4	Flavonoids	-	+	+
5	Carbohydrates	-	+	+
6	Alkaloids	+	+	+

Key: + = Present and - = Absent

Table 2: Antibacterial activity of mint, ajwain and tulsi using agar cup plate method

Natural Herbs	*Salmonella typhi*	*Escherichia coli*
	Zone of inhibition(mm)	Zone of inhibition(mm)
Mentha piperita (mint) 10% extract 20% extract	10 14	13 16
Ocimum tenuiflorum (Tulsi) 10% extract 20% extract	9 11	12 14
Trachyspermum ammi (Ajwain)	Nil	Nil

extract was more effective in inhibiting the growth of test bacteria as compared to 10% methanolic plant extract. 10% methanolic extract of mint showed 10 mm of zone of inhibition against *Salmonella typhi* and 13 mm of zone of inhibition against *Escherichia coli* while 20% methanolic extract of mint showed 14 mm of zone of inhibition against *Salmonella typhi* and 16 mm of zone of inhibition against *Escherichia coli.* 10% methanolic extract of tulsi showed 9 mm of zone of inhibition against *Salmonella typhi* and 12 mm of zone of inhibition against *Escherichia coli* while 20% methanolic extract of tulsi showed 11 mm of zone of inhibition against *Salmonella typhi* and 14 mm of zone of inhibition against *Escherichia coli.*

Discussion

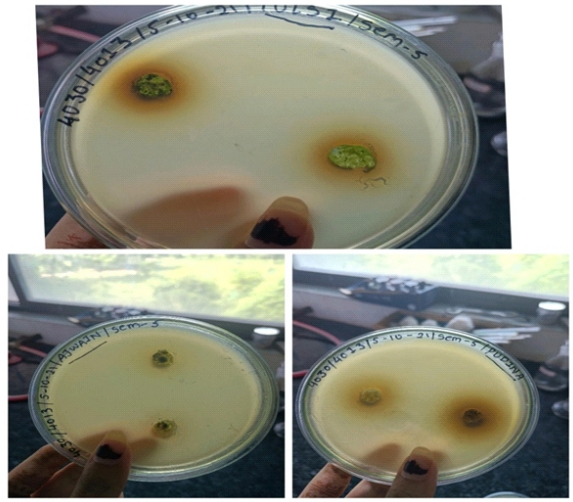

Figure 1: Growth inhibition of *E.coli* caused by Tulsi, ajwain and mint

The metabolites: alkaloids, steroids, saponins and tannins found in extracts are known to have curative activity against several pathogens and therefore Ajwain is use traditionally in Indian system of medicine to treat amoebiasis, febrile conditions, stomach disorders, dyspepsia and disorder of inflammation (Sreemoyee *et al.* 2012). Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavangers, anti-inflammatory and anti-carcinogenic. Due to presence of phenolic compounds these might play role in the prevention of several chronic diseases such as cardiovascular disease, cancer, diabetes, bacterial and parasitic

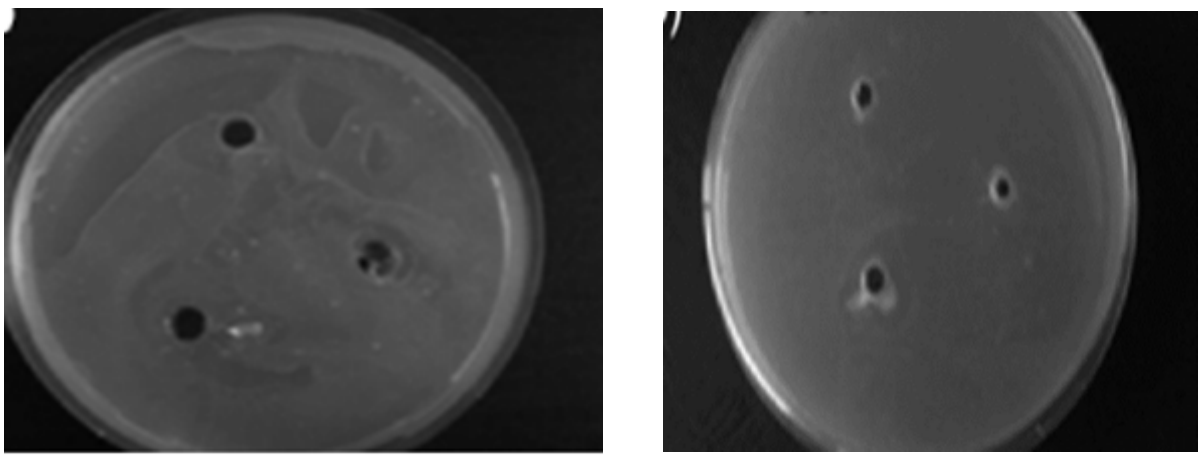

Figure 3: Effect of water, 10% methanol and 20% methanol on growth of *E.coli* and *S.typhi*

infections (Cazarolli *et al.* 2008). In this study ajwain showed no antibacterial activity. However, contrasting results hve been reported by conducted by Hafeez *et al.* (2018) it was observed that Ajwain essential oil showed antibacterial activity against *E. coli* showing 40 mm of zone of inhibition. Positive results were obtained in antibacterial activity of ajwain against pathogens according to previous studies and researches. There could be several reasons behind this negative result. The altitude or place from where the plant sample was collected could be one of the prominent reasons. The phytochemical compositions of plant may differ according to the altitude, place. Furthermore, the impurity in extract may also have led to the negative result of antibacterial test of leaves of *Trachyspermum ammi*. In addition to these, *Trachyspermum ammi* could/may have shown antibacterial activity, if extract was prepared in other solution beside methanol. In one study it was shown that methanolic extract of mint had no antibacterial effect on *E.coli* and *S. typhi* which is quite opposite to this study (Khanal *et al.* 2019). Similar to this studies Tulsi essential oil was found effective against *E. coli* showing 12 mm zone of inhibition (Helen *et al.* 2011).

Conclusion

According to this study, tulsi and mint are superior to ajwain at combating harmful bacteria like *Salmonella typhi* and *Escherichia coli*. The presence of phytochemicals in methanolic extract of leaves of plants justifies the pharmaceutical significance of all three plants. The proliferation of numerous dangerous bacterial strains frequently results in food deterioration and a number of other ailments. Chemical preservatives are mostly used in the food industry and food products to prevent food from spoiling. The negative health impacts of these chemical food preservatives drive up the desire for natural, safe, and potentially effective alternatives. These plant extracts can be used as all-natural preventives to manage food poisoning and other illnesses brought on by *S. typhi* and *E. coli*.

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