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In-vitro Efficacy of Methanol and Acetone Extracts of *Trigonella foenum* (Fenugreek) and *Trachyspermum ammi* (Ajwain) Against Pathogenic Bacteria

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Abstract: ***Spices are some of the most commonly used natural antimicrobial agent in food. Addition of spices in food not only imparts flavor and pungent stimuli but also provide antimicrobial property .Current research on natural molecules and product primarily focuses on plants since they can be sourced more easily and be selected on their ethano-medical properties .While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local use .Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases . The aim of the present study was to screen the medicinal and antibacterial activities of methanol and acetone extracts of the two spices Fenugreek (Trigonella foenum) and Ajwain (Trachyspermum ammi) available in Chhattisgarh .Crude extract of the spices with methanol and acetone were screened for antimicrobial activities against four pathogenic bacteria – Escherichia coli , Pseudomonas sp., Salmonella sp. and Staphylococcus aureus. In-vitro antibacterial activity was performed by agar well diffusion method. Methanol extract of Fenugreek and Ajwain revealed an elevated antimicrobial activity against Pseudomonas sp., Salmonella sp. and Staphylococcus aureus whereas acetone extract of spices exhibited highest activity against Escherichia coli. and showed little activity against Salmonella sp. The results obtained in the present study suggest that the methanol extract of Trigonella foenum and Trachyspermum ammi revealed a significant scope to develop a broad spectrum of antimicrobial herbal formulation.***

Keywords: Pathogenic bacteria, Spices, Antimicrobial activity, Fenugreek, Ajwain

1. Introduction

When recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition boosting flavor, herbs and spices are also known for their preservative and medicinal value [1] which form one of the oldest science.

Spices and herbs have been used for thousands of centuries by many cultures and scientific experiments have documented the antimicrobial properties of spices. Present investigation was planned to confer the antibacterial nature of two spices – Fenugreek / Menthi (Trigonella foenum) and Ajwain (Trachyspermum ammi), commonly used and cultivated in this region, also known for its medicinal value. Trigonella foenum L. commonly called as Fenugreek belongs to the family Leguminosae which is annual, herbaceous and aromatic plant. Fenugreek seed have been shown to exhibit strong inhibitory antimicrobial effects [2]. This is due to strong chemical i.e. diosgenin. The seeds of fenugreek are reported to have antidiabetic, antifertiliy, anticancer, antimicrobial and lactation stimulant. In Ayurveda both Fenugreek seeds and leaves are used to prepare extracts or powder for medicinal use [3]. Trachyspermum ammi L. commonly called as Ajwain belongs to the family Apiaciae which also annual, herbaceous and aromatic plant. Ajwain seeds contain much health's benefiting chemical compound such as thymol, a monoterpene derivative. Ajwain seeds are reported to have carminative, analgesic, anti-inflammatory, anti-oxidant and anti-septic properties.

Spices have been a major source of new drugs [4]. So many plants are used medicinally in different countries and are source many potent and powerful drugs. Medicinal plants are used by 80% of the world population as the only available medicine especially in developing countries [5]. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethano-medicinal uses [6]. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for the local used and traded in the market as the raw material for many herbal industries [7]. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [8]. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compound [9].

2. Materials and Methods

2.1 Plant materials:

Fresh, healthy plants and dried seeds of Fenugreek and Ajwain were collected from nearby fields and the market respectively. The sample specimen plants were botanically identified based on the taxonomical characteristics. The leaves of specimen plants were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves and seeds were subsequently dried in hot air oven at 40oC for 48hrs, powdered and used for extraction.

2.2 Preparation of extracts:

Ten gram of powdered leaves and seeds of Fenugreek and Ajwain was weighed and transferred into two separate 100 ml conical flask. Then 40 ml of methanol and acetone in both flasks were added. The conical flask were closed by foil paper and put on dark place for 24 hours. The crude extracts in both solvent were then filtered by passing the extract through Whatman filter paper No.1 and then concentrated under vacuum at 40 o C by using a rotary evaporator. Some amounts of concentrated extracts were used for further studies to assess its antibacterial properties.

2.3 Test organism:

Antibacterial activity of spices powder extracts was investigated against four pathogenic bacteria –Escherichia coli, Pseudomonas sp., Salmonella sp. and Staphylococcus aureus, which were obtained from recognized pathological laboratory. The borrowed bacterial cultured were maintained on Nutrient agar medium (Hi media, Mumbai) at 37 0 C for 24 h.

2.4 Antibacterial Activity:

The antibacterial activity was performed by agar disc diffusion methods [10]. Circular paper disc were cut from Whatman No.1 filter paper using a paper perforator. The discs were saturated with each of the reconstituted plant extracts, allow drying and were placed firmly (with the use of sterile forceps) on the surface of seeded nutrient agar plate. The plates were incubated for 24-48 h at 37 0 C. After incubation, the Zone of inhibition around the discs was measured by millimeter scale. Discs were impregnated with each treatment and control was assayed on duplicate agar medium plate for the test organism. The diameter of ZOI (mean of three replicates ± SD) as indicated by clear area which was devoid of growth of microbes was measured to determine the antibacterial activity. The experiment was replicated three times to confirm the reproducible results. The results obtained were compared with the standard antimicrobial agent - Cefixime (2µg/ml).

3. Results and Discussion

During investigation it was noticed that various extract of methanol were found more sensitive to bacterial isolates rather than acetone extracts. Crude extracts of both Fenugreek and Ajwain showed variable zone of inhibition (ZOI) against pathogenic bacterial strains (Table 1 & 2). Methanol extracts of Fenugreek seeds showed highest inhibitory effect against Pseudomonas sp., Salmonella sp. and Staphylococcus aureus. Fenugreek seeds and leaves extract showed wide spectrum activity, whereas acetone extract of spices exhibited highest activity against Escherichia coli and showed little activity against Salmonella sp. (Fig. 1 & 2) Ajwain seed is highly lethal to both Staphylococcus and Salmonella species. in comparison to other strain (Fig – 1 & 2). After bioassay extracts were found more or less susceptible to both Gm –ve and Gm +ve bacteria. The result obtained in the present investigation were based on crude extract however, suitable bacterial bioassay have been established to recognized and quantify antibacterial effect of different extract.

Table 1: Antibacterial properties of crude Methanol and Acetone extract of Fenugreek

Test Organisms	Zone of Inhibition (in mm.)				
	Leaves Extract		Seeds Extract		Antibiotic (Cefexine)
	Methanol	Acetone	Methanol	Acetone	
E. coli	8 ±0.71	9 ±0.41	10 ±0.36	11 ±0.26	17 ±0.03
Pseudomonas sp.	10 ±0.25	4 ±0.52	13 ±0.38	5 ±0.67	18 ±0.05
Salmonella sp.	10 ±0.50	3 ±0.64	13 ±0.45	4 ±0.72	17 ±0.07
Staphylococcus aureus	11 ±0.62	5 ±0.28	14 ±0.50	6 ±0.55	19 ±0.12

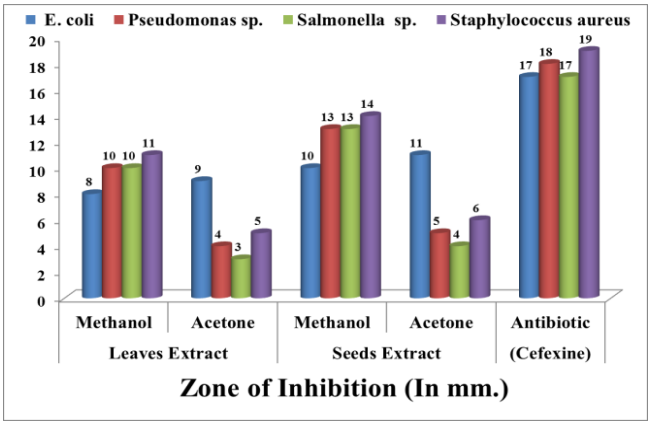

Figure 1: Antibacterial properties of crude Methanol and Acetone extract of Fenugreek

Table 2: Antibacterial properties of crude Methanol and Acetone extract of Ajwain

Test Organisms	Zone of Inhibition (in mm.)				
	Leaves Extract		Seeds Extract		Antibiotic (Cefexine)
	Methanol	Acetone	Methanol	Acetone	
E. coli	8 ±0.64	7 ±0.53	10 ±0.45	9 ±0.36	17 ±0.03
Pseudomonas sp.	7 ±0.32	3 ±0.44	8 ±0.28	4 ±0.46	18 ±0.05
Salmonella sp.	8 ±0.46	2 ±0.47	9 ±0.25	3 ±0.58	17 ±0.07
Staphylococcus aureus	9 ±0.52	3 ±0.38	9 ±0.53	4 ±0.52	19 ±0.12

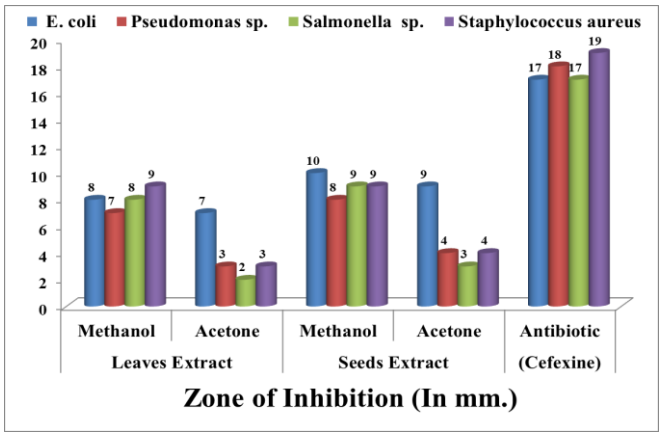

Figure 2: Antibacterial properties of crude Methanol & Acetone extract of Ajwain

4. Conclusion

The extracts of Trigonella foenum L. and Trachyspermumm ammi were found to be effective antibacterial agents against human pathogen. Bio-molecules of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogen. This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect.

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