In vitro Antibacterial activity of *Pimpinella anisum* fruit extracts against some pathogenic bacteria

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Abstract

The antibacterial activities of the aqueous, 50% (v/v) methanol,acetone and petroleum ether extracts of *Pimpinella anisum* (L) fruits were studied. The extracts of *Pimpinella anisum* were tested *in vitro* against 4 bacterial species by the disc diffusion method. *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherchia coli* (MTCC 723) and *Klebsiella Pneumoniae* (MTCC 109) were used in this investigation. Only aqueous and 50% (v/v) methanol extract exhibited fair antibacterial activity against all the test bacteria whereas acetone and petroleum ether extract were not observed to inhibit the growth of any of the test bacteria under study.

Keywords: Antibacterial Activity, Pimpinella anisum, Pathogenicity, Bacteria

Introduction

Pimpinella anisum is a flowering plant in the family Apiaceae, native to the India and southwest Asia. It is a herbaceous annual plant growing to 1m tall. The leaves at the base of the plant are simple, 2-5 cm long and shallowly lobed, while leaves higher on the stems are feathery pinnate, divided into numerous leaflets. The flowers are white, 3 mm diameter, produced in dense umbels. The fruit is an oblong dry schizocarp, 3-5 mm long.

As a medicinal plant, *Pimpinella anisum* has been used as a stimulating effect of digestion and antiparasitic, antifungal (Soliman and Badea, 2002) and antipyretic (*Afifi,et.al.,*1994). Additionally, the plant and especially its fruit essential oil have been used for treatment of some disease including seizures and epilepsy (Avicenna,1988;*Abdul-Ghani,et.al.,*1987). Furthermore, it has been shown to have anticonvulsant effects and has been used for the treatment of constipation (*Curtis,et.al.,* 1996; *Pourgholam,et.al.,* 1999; Chicouri and Chicouri, 2000) and possesses muscle relaxant effect (*Albuquerque,et. al.,*1995).Recently its oil has been reported to be used as antibiotic substitute in broiler ration (*Mehmet,et.al.,*2005).

There are few reports (*Singh,et.al.,*2002; *Tabanca,et.al.,*2003) on systematic studies pertaining to antibacterial evaluation of *Pimpinella anisum.* Hence, considering its therapeutic potential, it was essential to prove it for its exact rational use as medicine by scientific means. Therefore, the present investigation was undertaken to evaluate antibacterial activity of *Pimpinella anisum* dried fruits against some pathogenic bacteria.

Material and Methods

Fruits collection: The fruits of *Pimpinella anisum* were purchased from the local market from three different sources. The materials were verified from Department of Botany, Maharashtra Udaygiri College, Udgir, district Latur, Maharashtra.

Extraction: The fruits were shade dried and ground to powder form using the grinder mixer. Fifty gram dried fruits powder was soaked separately for 48 hrs in 200ml distilled water, 50% (v/v) methanols, acetone and petroleum ether for aqueous, alcoholic, acetone and petroleum ether extraction respectively. The soaked material was agitated at regular time intervals. After 48 hrs the soaked material was filtered using muslin cloth .Then the filterate were again filtered using whattman filter paper No 1. on separate filtration setups. The final filterates were collected in wide mouthed evaporating bowls and dried under room temperature. The dried extracts were weighed to calculate the extractability percentage. The extracts were stored at 40 C until further use.

Bacterial panel: *Pimpinella anisum* fruits extracts were tested for antibacterial activity using a panel

1. M.V.Sc. Students 2. Professor and Head 3. Assistant Professor 4. B.V.Sc & A.H student

of *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherchia coli* (MTCC 723) and *Klebsiella Pneumoniae* (MTCC 109).The organism were cultured and maintained on nutrient agar (MM012) and in Nutrient broth (M088).

Antibiotic assay: Assays were performed as previously described (*Bauer, et.al.,*1966) with some modifications. In brief; each extract was pipetted onto a sterile paper disc (*Hi Media Laboratories Ltd.*, Mumbai, India) that had been suspended on top of a dissecting needle. The volume added was chosen because it represents the approximate volumetric capacity of each disc. The solvent was allowed to evaporate and the discs (upto 9 plate-1) were then placed onto the surface of Petri dishes that had previously been surface-inoculated with individual test strains at 10^6 dilution. Plates were then incubated at 37°C for 24 hrs. Solvent control discs were prepared in the same manner and were never observed to inhibit bacterial growth. Cipro-floxacin discs, treated with 30 mg disc-1 of ciprofloxacin (*Hi Media Laboratories Ltd.*, Mumbai), were used as antibiotic standards on each plate. Three replicate extracts, each from a sample of the same species of *Pimpinella anisum* collected from three different sources, were assayed on different plates.

After incubation, the diameter of clear zone surrounding the disc was measured to the nearest mm. An extract was considered active if one or more replicate extracts (each from a different fruit sample of the same species, but from different sources) produced a zone of inhibition $\geq$1 mm beyond the edge of the disc. The area of this zone of inhibition was calculated for each replicate assay of discs treated with extracts, and for ciprofloxacin discs, and mean and standard deviations calculated for all treatments. Because the disc diffusion assay is used to assess qualitative differences in antimicrobial activity, and is not without limitations (*Jenkins, et. al.,*1998), statistical analyses were not used to assign significance between treatments.

Results and Discussions

The percent extractability was highest for aqueous (18.16%) and lowest for petroleum ether (7.51%). The percentage extractability for 50% (v/v) methanol and Acetone were 12.22% and 9.08% respectively. (**Table.1**).

Table1. Extractability Percentage of various solvent extracts

Extract/Drug	Extractability %
Aqueous	18.16
Acetone	9.08
Petroleum Ether	7.51
Methanol	12.22

The amount of extract present on the disc prepared in Aqueous, Acetone, Petroleum ether, and 50%(v/v) Methanol extract were 22.43±0.35, 8.66±0.26,9.66±0.70 and 19.04 ± 0.74 respectively. The amount of extract impregnated on the disc was highest in aqueous and lowest in petroleum ether (**Table 2**). Therefore the amounts of different extracts impregnated on the discs were proportional to the percent extractability of the respective extract.

The aqueous extract was found to be effective against all the pathogenic bacteria under test by disc diffusion assay. It exhibited fair antibacterial activity against all the test bacteria. The petroleum ether and acetone extract failed to exhibit any zone of inhibition by disc diffusion assay. The 50% (v/v) methanol extract was found slight less effective than aqueous extract against all the pathogenic bacteria under test by disc diffusion assay. The reference drug (Ciprofloxacin) was more effective than all the *Pimpinella anisum* fruits extracts against all the pathogenic test bacteria when studied by disc diffusion assay (**Table 3).**

The aqueous extract was found to be the most potent antibacterial extract, also the activity against both gram(+ve) and gram(-ve) bacteria suggest its potential use as a broad spectrum antibacterial agent. Further considering the cost, availability and extractability percentage of the aqueous extract, it can be considered & used as a cheap alternative to substitute antibiotics, especially in animal and poultry feeds.

Table2. Amount of different extracts impregnated on the discs

Extracts	Weight of Blank disc	Extract impregnated on disc	Extract in each disc
Aqueous	13.00± 0.00	35.42 ± 0.64	22.43 ±0.35
Acetone	13.00± 0.00	21.66 ± 0.42	9.66 ± 0.70
Petroleum ether	13.00± 0.00	22.26 ± 1.26	8.66 ± 0.26
Methanol	13.00± 0.00	32.04 ± 1.10	19.04 ± 0.74

Table3. Zone of inhibition of different extracts of fruit of plant *Pimpinella anisum* and reference drug against different bacteria

Extract/ Drug	Mean Diameter ± S.E (mm)			
	E. coli	*S.aureus*	*S. pyogenes*	*K.Pneumoniae*
Aqueous	12.00±0.12	13.00±0.24	12.00±0.31	13.00±0.34
Acetone	No Zone	No Zone	No Zone	No Zone
Petroleum Ether	No Zone	No Zone	No Zone	No Zone
Methanol	11.00±0.11	8.00±0.12	11.00±0.20	9.00±0.12
Ciprofloxacin	34.00±0.24	33.00±0.24	30.00±0.14	28.00±0.47

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Camphylobacteriosis from consuming unpasteurised milk in California, USA

Alexandre Family EcoDairy Farms ended its raw milk supply after several people who consumed the product got sick, including a Crescent City woman who remains in intensive care and is partially paralyzed. The Del Norte County Department of Public Health suspects at least 15 people who ingested raw milk contracted campylobacteriosis, a common bacterial infection found in domesticated animals that can cause gastrointestinal illness in people. Raw milk essentially comes straight from the udder and has become popular among health-conscious consumers. It is unpasteurized, and advocates say it contains beneficial microbes that help in digestion and provide increased nutrition. About 115 people were signed up for Alexandre EcoDairy's raw milk supply program.

It is illegal to sell raw milk in California , but it is not illegal to get it from your own animal. The Alexandres devised a cow-share program that allowed people to buy stock in an Alexandre cow. That gave them personal ownership of the animal and allowed them to legally take raw milk from Alexandre EcoDairy. Before customers could join the cow-share program and get the raw milk, Alexandre gave them a 3-ring binder full of information. In the 1st section are a number of articles relaying the dangers of consuming raw milk. To join the cow-share program, customers signed an agreement relieving the eco-dairy of liability in case of health problems caused by raw milk. Raw milk is a well-documented source of infections from Salmonella, E. coli O157:H7, Campylobacter, Listeria, Mycobacterium bovis, and other pathogens.

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