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Antibacterial activity of some Medicinal Plants used against UTI causing Pathogens

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Abstract

It was a study to investigate the Antibacterial Activity of Some Medicinal Plants Used against UTI Causing Pathogens. Bacteria were isolate from the UTI infected patient and characterized by using microscopic, staining, morphological and biochemical methods. Oils from plants were extracted using Clevenger and these oils were than used to check their antibacterial activity against the bacteria isolated from UTI infected patients and the zone of inhibition were compared with the zone of inhibition of standard antibiotics. Results from the present study showed that ajwain oil had more antibacterial activity compared to other oils we used and Fennel oil had lowest antibacterial activity.

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INTRODUCTION

A urinary tract infection (UTI) is an infection that begins in the urinary system. It is the second most common disease after respiratory infection. The urinary tract consists of the kidneys, ureters, bladder and the urethra [6]. More than 95% of UTI are caused by single bacterial species *E. coli* which is the most frequently infecting organisms [9]. However, many other bacteria can also because an infection for example, *Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus, Mycoplasma, Chlamydia, Serratia* and *Neisseria spp*. It is reported that about 35% of healthy women suffer symptoms of Urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria) and frequency [7]. The incidence of UTI is greater in women as compared to men. Several potent antibiotics are available for the treatment of UTI, but

increasing drug resistance among bacteria has made therapy of UTI difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs [14]. Essential oils and extracts of certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods [4]. The synergistic effect of the mixture of phytochemicals play important role to use plant extracts as antimicrobial agents [11]. It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels [3]. The antimicrobial and other biological activities of the essential oils varied depending upon the origins and cultivars [8]. In this study antimicrobial activity of the oils was checked against the bacterial isolates from UTI patients, 5 different spices cinnamon; peppermint, fennel, ajwain, clove oil were select to extract oil for antimicrobial activity.

MATERIAL AND METHODS

Isolation and identification of bacteria from UTI infected patients:

First of all microorganism present in urine samples of UTI infected patients were cultured in the nutrient broth and after it the morphology of organisms were studied with the help of light microscope and shape, size, odour, margin and surface characteristics of bacteria were studied. Gram staining procedure was adopted to differentiate between gram positive and gram negative organisms.

Selective agar medium was used for further identification.

Different media for different organism were used as given below:

- Mac Conkey : *E. coli* and *Staphylococcus*
- EMB : *E. coli*
- MSA : *Staphylococcus*
- PABM : *Pseudomonas*
- XLDA : *Shigella*
- Blood agar : *Proteus*

After this step final identification of bacteria was done on the bases of biochemical testing.

BiochemicaL Tests:

Seven biochemical tests were performed for each organism as given below:

- Catalase test
- Indole production test
- Citrate utilization test
- TSI (triple sugar iron) agar test
- Urease activity test
- MR and VP test

Extraction of Volatile Oil:

Five spices were preferred for which antimicrobial activity was being to be tested named as:

- Ajwain oil : Trachyspermum copticum
- Cinnamon oil : Cinnamomum zeylanicum
- Clove oil : Eugenia caryophyllus
- Fennel oil : Foeniculum vulgare
- Peppermint oil : Mentha pipperita

250 gm of each spice was taken and poured into clavenger apparatus in the lab with the addition of water (temp.) the extracted oils were collected in the air tight container (volatile) for further use.

TESTING OF ANTIMICROBIAL ACTIVITY:

Testing the antimicrobial activity by well diffusion method:

Muller Hinton agar was use to check antimicrobial activity by well diffusion method.

Autoclaved medium was poured in to petriplates in the laminar air flow hood. On cooling the medium within petriplates the microorganism from 24 hrs old broth were spread then wells were made on the petriplates with the help of stainless steel borer of diameter 6- 8 mm. Three wells were made on entire surface of medium; one is for standard antibiotic to the test organism and remaining two for different oils.

Two wells were also made on the entire surface at angle 180°, one is for specific antibiotic to the test organism and one for ajwain oil because this oil has showed very large zone of inhibition in the previous trial.

Now 100 micro-liter of standard antibiotic was poured in the first well and 100 micro-liter of the different oil in remaining two wells.

These plates were incubated for 24 - 48 hrs and the diameter of zone of inhibition was measured with the help of scale

RESULTS & DISCUSSION

After studying the colony morphology on nutrient agar medium, colony morphology was also studied on the selective media. After the secondary identification on selective media, all samples were examined microscopically here the shape size arrangement (pair, cluster and chain) and motility was checked and the gram staining technique are followed.

After the steps above final identification were done on the basis of biochemical analysis we isolated five types of bacteria (*Staphylococcus, E .coli, Proteus, Shigella,* and *Pseudomonas*) from different samples of urine of UTI infected patients. The most common pathogens isolated in these pregnant women were *E. coli* (37.0%), *Klebsiella spp*. (20.4%), *P. mirabilis* (16.7%), *P. aeruginosa* 7 (13.0%), *S. aureus* 4 (7.4%) and *S. epidermidis* (5.6%). This finding is similar to other reports which indicate that Gram-negative bacteria, particularly E. coli is the most implicating pathogen isolated in patients with UTIs [1].

The results of final identification on the basis of biochemical analysis are shown in the Table 1.

Table1: Biochemical tests of recovered isolates of urine samples from different hospital and clinical labs.

S. No	catalase	indole	MR	VP	TSI	Citrate	urease	Gas production	Organism confirmed
1	+	+	+	-	+	-	-	+	E.coli
2	+	-	+	+	+	-	-	+	Staphylococcus
3	+	+	+	-	-	-	-	-	Shigella
4	+	+	+	-	+	-	-	+	E.coli
5	+	-	+	+	+	-	-	+	Staphylococcus
6	+	+	-	-	+	+	+	+	Pseudomonas
7	+	+	-	-	+	+	+	+	Pseudomonas
8	+	+	+	-	+	+	+	+	Proteus
9	+	+	+	-	-	-	-	-	Shigella
10	+	+	+	-	+	+	+	+	Proteus
11	+	+	+	-	+	-	-	+	E.coli
12	+	+	+	-	+	+	+	+	Proteus
13	+	+	+	-	+	-	-	+	E.coli
14	+	-	+	+	+	-	-	+	Staphylococcus

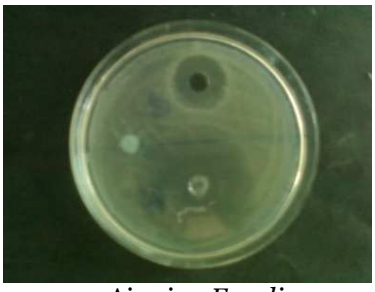
Antimicrobial Activity

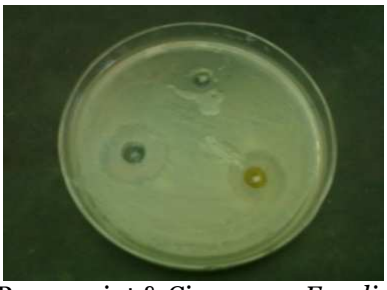
Five oils of the spices cinnamon, clove, ajwain, peppermint and fennel were extracted to test the antimicrobial activity on the five different bacteria isolated from urine sample of UTI infected patients with respect to standard antibiotic by the “agar well diffusion method” and the diameter zone of inhibition was measured in mm.

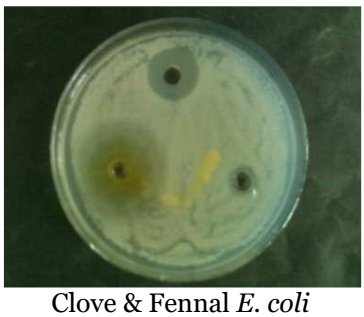
Antimicrobial activity of different oils on the different organisms is given as in Table 2.

Table 2: Diameter of zone of inhibition shown by various isolates against standard antibiotic and various oils extracted from spices.

Organism	**Cinnamon** (zone of inhibition in mm)	**Clove** (zone of inhibition in mm)	**Ajowin** (zone of inhibition in mm)	**Fennel** (zone of inhibition in mm)	**Peppermint** (zone of inhibition in mm)	**Antibiotics** (zone of inhibition in mm)
Staphylococcus	23	30	31	11	17	Gentamycin 19
E.coli	20	26	46	12	26	Norfloxacin 22
Proteus	25	19	38	15	17	Ciprofloxacin 25
Shigella	26	26	35	10	23	Norfloxacin 23
Pseudomonas	26	28	32	12	21	Norfloxacin _

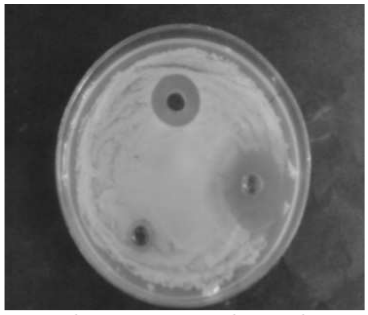

Ajwain *E. coli*


Peppermint & Cinnamon *E. coli*

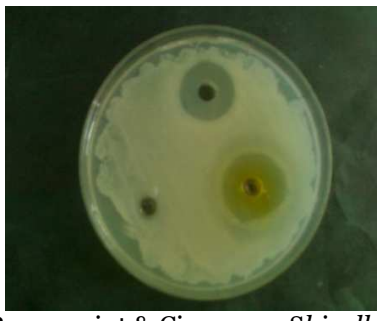

Clove & Fennal *E. coli*

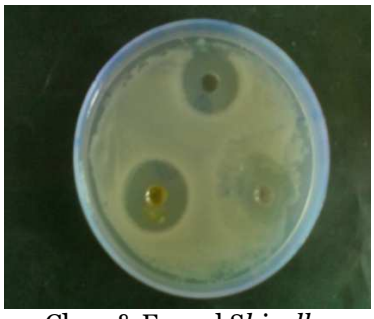

Ajwain S*taph.*


Peppermint & Cinnamon S*taph.*

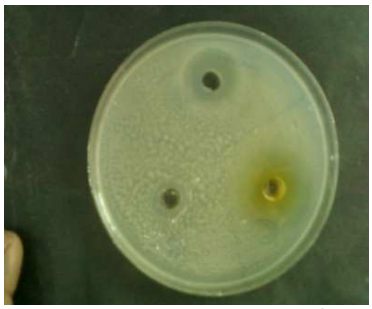

Clove & F*ennal Staph.*


Ajwain *Shigella*


Peppermint & Cinnamon S*higella*


Clove & Fennel S*higella*


Ajwain P*seudomonas*


Peppermint & Cinnamon P*seudomonas*


Clove & fennel P*seudomonas*

Figure1: Showing zone of Antimicrobial activity of different oils on the different bacteria

Results showed that Ajwain oil has greater anti bacterial activity among all the oils its maximum value of the zone of the inhibition is noted against *E. coli* 46mm approximately 2.1 times than norfloxacin 22mm. Then clove oil and cinnamon oil has showed almost equal activity, clove oil has maximum activity against *Staphylococcus* with diameter 30mm in comparison to gentamycin 19mm. Cinnamon oil had showed maximum results of antimicrobial activity against the organisms *shigella* and *Pseudomonas* 26mm in comparison to norfloxacin 23mm and 20mm. After that peppermint oil has showed almost equal results to the antibiotics with maximum activity 26mm against the bacteria *E. coli* in comparison to norfloxacin 22mm and the Fennel oil has show least activity of all the oils and also less than the standard antibiotic maximum zone of inhibition 15mm in comparison to standard antibiotic ciprofloxacin 25mm. Zone of Inhibition given by some medicinal plants were also obtained by [12] in different bacterial strains such as *Pseudomonas aeruginosa* 33.3 mm, B. subtilis 29.9 mm P. vulgar is 29.4 mm, *K. pneumonia* 20.8 mm and *S. aureus*. Besides this, some plant essential oils have shown growth inhibitory effets against Clostridium ferfringens, *E. coli* and Lactobacillus acidophilus [5], Bacillus species [10], *Staphylococcus aureus* [2], *Solmonella enteritidis* [13].

CONCLUSION

From the above results we can conclude that oil of plants origin has remarkable antimicrobial activity as compare to antibiotic activity. We know that organisms are gaining resistance day by day towards the antibiotics, so that some natural product should be try to overcome these antibiotic resistant organisms. Moreover plants oils have no side effect; volatile oil may be one of our choices because oil contains hydrophobic liquid which can be easily extracted by the process of distillation. Oils contain volatile aroma and phytochemicals which show the antimicrobial activity. More over plants can be grown easily and the production of oils is sophisticated than antibiotic Expense on these material is bearable than antibiotic. From these properties of oils we can say that natural medicine can take place of antibiotics in future.

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