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Antibacterial Activity of Some Plant Extracts Against Multidrug-Resistant Pathogenic *Escherichia Coli.*

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ABSTRACT

In this study, we investigated the in-vitro effect of 11 medicinal plants' methanolic extracts against 3 strains of multidrug resistance *E coli* which were confirmed via the VIETK2 system and were given numbers (1, 2, 3) to distinguish each one.

Strain 1and 3 showed complete resistance to 8 different types of antibiotics, while strain 2 showed 11 types of antibiotics out of 11 types of antibiotics that were used in this study.

Strain 1 showed sensitivity towards extracts of (Green tea, Fennel seed, Pomegranate peel), strain 2 showed sensitivity towards (Fennel seed, Sage leaves, Thyme leaves) extracts, and strain 3 showed sensitivity towards (Green tea, Fennel seed, Pomegranate peel, Sage leaves, Thyme leaves).

INTRODUCTION

In recent years, Various *Escherichia coli* bacteria species have developed resistance to antibiotics (Palaniappan & Holley 2010). this resistance has been existed due to a lot of reasons, but the most important reason has been the inappropriate consumption of antibiotics, whether by unnecessary use or by consuming wrong doses or types (Jastaniah,2014).

Knowing that *Escherichia coli* bacteria is the most common causative organism that is responsible for nosocomial infectious diseases in humans (Sligl *et al*., 2006).

It is known that at least there are 5 pathotypes of different *E coli* bacteria that cause enteric diseases such as bacterial dysentery and diarrhea (Kokoska *et al*., 2002) also various types of extra gastric diseases like meningitis and Urinary tract infection (Kokoska *et al*., 2002). So researches were going in the direction of finding a new drug that could affect multidrug-resistant bacterial strains, and due to the good reputation of the folkloric remedies that are based on medicinal plant extracts or their derivatives against infectious diseases, so medicinal plants has become a respectable alternative to antibiotics in fighting multidrug-resistant *Escherichia coli* a lot of studies have been putting medicinal plants on research to get authentic results, and a lot of discovered secondary medicinal products that hold antibacterial properties were discovered and proved their efficiency in fighting MDR bacteria. (Mahmoud *et al*., 2004).

Escherichia coli is a bacilli gram-negative non-forming spores, flagellated and facultative anaerobic bacteria, from the family Enterobacteriaceae (Kaper *et al*., 2004).

E. coli is exciting in non-pathogenic form as normal flora of the human lower intestine as a commensal bacterium, in this form it causes no harm to human, but when it be found where else it develops virulence factors and become in its pathogenic form and cause disease in the place it was found in (Jain *et al*., 2005)

E. coli bacteria are lactose fermenting bacteria, it grows perfectly on Maconcky media and produces indole, it also gives a positive result in the catalase test and a negative result in the oxidase test, and some e coli subtypes ferment sorbitol (Kaper *et al*., 2004).

There are pathogenic 5 *E. coli* subtypes that are responsible for enteric diseases: enterotoxigenic *Escherichia coli* (ETEC), enter invasive *Escherichia coli* (EIEC), enteroaggregative *Escherichia coli* (EAEC), enteropathogenic *Escherichia coli* (EPEC), and, enterohemorrhagic *Escherichia coli* (EHEC), which also called Shiga toxin-producing *Escherichia coli* (STEC) which also could be referred to as EHEC/STEC (Nataro & Kape, 1998).

They are been differentiated from each other by their O antigen which is located on the lipopolysaccharide outer membrane, H antigen which is located on the flagellum (Nataro & Kape, 1998).

MATERIALS AND METHODS

In this study, we used eleven plants, plants were chosen based on their reputation by having a therapeutic effect against infectious diseases, different parts of each plant were used according to each plant folkloric way of preparation, plants parts were washed gently by distilled water and dried by fresh air at room temperature for three days, then each plant parts were carefully grounded by (Siemens-blender), After grounding, 10 g of each plant powder was weighed, placed in a sterilized Falcon tube and 50 ml of methanol were added to each Falcon tube (Aneja *et al*., 2010).

Falcon tubes were covered tightly by their plastic lids, put in a shaking incubator for 48 h, and then each supernatant was collected and was filtered through three layers of filtration paper then centrifuged at 5000 rpm for 10 min, each extract was concentrated to only 5 ml in Vacuum via Heidolph VE-11 Rota evaporator below 40^{o}C. The final products were stored in sterilized containers at 4^{o}C degrees. (Aneja *et al*., 2010).

Studied Bacteria:

Three *E. coli* bacterial strains were obtained from three different clinical specimens (urine, stool, sputum) collected from three different patients who were getting treatment in Benha Educational Hospital.

Every specimen was cultured on selective Macnky agar media and was identified as *E. coli* bacteria by Gram stain, morphological and biochemical tests. Then each strain was purified on nutrient agar media.

Antibiotic Sensitivity Test:

Studied bacterial strains were tested against 11antibiotics, by the Disk diffusion method, those Antibiotics were:

1. Meropenem (MEM10 µg)
2. Gentamicin (GN10µg)
3. Moxifloxacin (MFX5 µg)
4. Tobramycin (TMN10 µg)
5. Levofloxacin (LVX10 µg)
6. Ofloxacin (OFX-5 µg)
7. Tetracycline (TS25 µg)
8. Trimethoprim+ Sulfamethoxazole (SXT 25 µg)
9. Ciprofloxacin (CIP5 µg)
10. Amoxicilin\Glavulanic acid (AuG 30µg)
11. Ceftriaxone (CTX30 µg) (CLSI), 2007)

Bacterial strains were cultured in Petri dishes with 20 ml of Muller Hinton agar media by swapping technique, and then antibiotic disks were placed and incubated at 37^{o}C for 24 h.

Plant Extracts Activity Test:

Each bacterial strain was tested against the 11 plant methanolic extracts by disk diffusion method, 1 ML of each extract was inoculated to sterile paper discs (6 mm

in diameter), and was put in a sterile petri dish to dry in a sterilized condition, bacterial strains were inoculated to Petri dishes with 20 ml of nutrient agar media by swapping technique then dried disks loaded with plant extracts were placed on cultures surfaces. then transferred to incubation at 37^{o}C for 24 h**.** (Cowan,2009)

Statistical Analysis:

One-way ANOVA was carried out using the statistical analysis system (SAS/STAT ® 9.1) according to the software procedure's guide (SAS,2004) Citation: SAS Institute Inc.2004.SAS/STAT®9.1 User's Guide. Cary, NC: SAS Institute Inc.

RESULTS AND DISCUSSION

Three *Escherichia coli* bacterial strains were isolated from three different specimens from 3 different patients and were given numbers (1, 2, 3) were identified as *Escherichia coli* bacteria, the three strains showed fermenting to lactose, positive catalase test negative oxidase test, grown on Mac agar media and produced indole.

All three bacterial strains were Gram-negative bacteria rod-shaped, formed white waxy colonies on nutrient agar media, and were confirmed as *E. coli* via VIETK2 system.

Antibiotics Sensitivity Test:

As shown in table 1, the three bacterial strains showed resistance to at least eight antibiotics out of eleven antibiotics, strain one showed resistance to nine antibiotics, strain two-showed resistance to ten antibiotics and strain three-showed resistance to eight antibiotics.

This antibiotic resistance could be developed by bacterial strains for various reasons, but the most important reason was antibiotic abuse whether by unnecessary use by patients or by consuming the wrong dose or type. knowing that bacteria is a prokaryotic organism, so genetic mutations that enable bacteria to adapt the environmental conditions are happening frequently and could be another reason for developing antibiotic resistance.

Antibacterial Activity of 21 Medicinal Plant Methanolic Extracts Against MDR *E coli*:

As shown in table 2, the result of 11 medicinal plant methanolic extracts against three different MDR *E. coli* bacterial strains, fennel seed methanolic extract showed antibacterial activity towards the three studied bacterial strains, but pomegranate peel showed the biggest inhibition zone on only two bacterial strains.

Table 1: Antibiotics sensitivity pattern of 3 MDR *E coli* strains tested by 11 antibiotics.

Antibiotic / Bacteria	MEM	GM	MFX	TMN	LVX	OFX	TS	CTX	CIP	AUG	SXT
Strain 1	S	S	R	R	R	R	R	R	R	R	R
Strain 2	S	R	R	R	R	R	R	R	R	R	R
Strain 3	S	R	R	R	R	R	R	S	R	S	R

Table 2: Antimicrobial activity pattern of three MDR *E coli* strains affected by (6mm) 11 disks loaded with 11 medicinal plant methanolic extracts

Plant name	Scientific name	Plant part	Strain 1	Strain 2	Strain3
Ginger	*Zingiber officinale*	root	N	N	N
Cinnamon	*Cinnamomum verum*	bark	N	N	N
Thyme	*Thymus vulgaris*	leaves, flowers	N	P **IhZ:**0.3 cm	P **IhZ:**0.4cm
Sage	*Salvia officinalis*	leaves, stem	N	P **IhZ:**0.4cm	P **IhZ:**0.2cm
Rosemary	*Salvia rosmarinus*	leaves, stem	N	N	N
Fennel	*Foeniculum vulgare*	bulb, leaves	P **IhZ:**0.4cm	P **IhZ:**0.5cm	P **IhZ:**0.4cm
pomegranate	*Punica granatum*	fruit peel	P **IhZ:**0.6cm	N	P **IhZ:**0.7cm
Green tea	*Camellia sinensis;*	leaves	P **IhZ:**0.3cm	N	P **IhZ:**0.2cm
Tumeric	*Curcuma longa*	root	N	N	N
clove	*Syzygium aromaticum*	flower buds	N	N	N
Black seed	*Nigella sativa*	seeds	N	N	N

IhZ: inhibition zone.

Conclusion:

This study aimed to find alternative materials that could affect multidrug-resistant *E. coli* bacteria which were confirmed as MDR after were tested by eleven antibiotics, bacterial strains were affected by methanolic extracts of eleven medicinal plants and found that fennel seed extract has affected all of the three strains, however, pomegranate fruit peel showed the most powerful extract with biggest inhibition zone. Fennel seed extract can be used as an effecting remedy against *E. coli* bacteria that no longer respond to Antibiotics or even a safe choice before trying antibiotics medication.

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