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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF CERTAIN MEDICINAL PLANT AND THEIR ESSENTIAL OILS ON THE ISOLATED BACTERIA FROM UTI PATIENTS.

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Abstract

In the current study, UTI bacterial isolates were then analyzed to determine their susceptibility profile to 30 antibiotics according to the standard CLSI guide. Among the tested samples, 919 bacteria isolated from urine samples (88.4%) were sensitive to the 30 tested antibiotics; 121 samples (11.6%) recognized as multi-drug resistant bacteria. The results also indicated that the most of urinary tract infection diseases were by Gram negative bacteria (102 isolates; 84.3 %).Additionally, in the present study among 15 plants extracted by boiled-water, ethanol or tested as essential oils, highest antibacterial activity was exhibited by essential oils plant extracts. Among all plant extracts highest IZ values were recorded for *Cinnamomum zeylanicum* in the range of 46.3 and 28.4mm against UTI bacterial isolates in following order *Staphylococcus aureus* > *Escherichia coli* >*Pseudomonas aeruginosa* >*Enterococcus faecalis* >*Klebsiella pneumoniae*. The next higher activity was observed for *Thymus vulgaris*, *Origanum majorana*, *Syzygium aromaticum*, *Zingiber officinale*, *Salvia officinalis* and *Rosmarinus officinalis* with the same inhibition pattern exhibited by *Cinnamomum zeylanicum*.Moreover, the ultrastructural effect of cinnamon essential oils on *Escherichia coli* and *Staphylococcus aureus* were also studied and showed dramatic cellular alterations on TEM electron micrographs with most of effects on bacterial cell wall.GC/MS analysis of essential oils indicated that cinnamon oils had fifteen components, and cinnamaldehyde was the major constituent (72.87%). On the other hand, the main components of marjoram oil were linalool (29.20%), trpenin-4-ol (20.04%) and γ-terpinen (11.89). However, thyme oil had 17 major components includes P-cymen (29.15%), thymol (24.80%) and carvecol (22.69%).

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Introduction:-

As the result of increasing mis-use and extensive uses of antimicrobial agents, bacterial pathogens have shifted away from easily treatable bacteria towards more resistant bacteria. This change is important problem for nosocomial infection control and prevention (**Mahdy *et al.*, 2012**).

The incidence of urinary tract infections by bacteria is problematic. However, a wide spectrum of treatment can be ranging from a single-dose antibiotic treatment, to rescue nephrectomy for pyonephrosis in diabetic patients with septic shock (**Kang *et al*., 2011**).

Medicinal plants produce a wide variety of secondary metabolites many of which have been reported to be of therapeutic value and a promising source of antibacterial compounds **(Rath *et al.*, 2012)**; raising hopes of obtaining novel antibiotics that can aid the fight against drug resistant infections.

Since several plant antimicrobials contain different functional groups in their structure, their antimicrobial activity is attributed to multiple mechanisms. Therefore, unlike antibiotics, the potential for bacteria to develop resistance to plant antimicrobials is relatively smaller **(Tepe *et al.*, 2004).**

According to World health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care needs **(Vashist** and **Jindal, 2012),** and Over 50% of all modern clinical drugs are of natural product origin **(Kumar** and **Chandrashekar, 2011).** Medicinal plants have been used in traditional medicine for the treatment of urinary tract disease. Interest in the folk medicine is increasing because many patients believed that such products are effective and less harmful **(Motlagh *et al*., 2013).**

The medicinal value of plants is related to their phytochemical components and their secondary metabolites **(Mohammedi** and **Atik, 2011).** Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids. The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenol **(Abubakar *et al.*, 2008).**

These natural products provide clues to synthesize new structural types of antimicrobial chemicals that are relatively safe to man **(Kalimuthu *et al*., 2010).** It is believed that crude extracts from some medicinal plants are more biologically active than isolated compounds due to their synergistic effects **(Soković *et al.*, 2010).**

Therefore, the current study aimed at testing three extracts from 15 medicinal plants to treat UTI bacterial isolates. Also, the effect of the effective essential oils on the ultrastructure of the bacterial cells was tested using TEM along with chromatographic characterization of the chemical constituents responsible for the activity.

Materials and Methods:-

Bacterial isolation and identification:-

Urine samples were collected from 1600 patient's (males, and females) of different ages from in patients and out patients of two hospitals: Al-Zahraa University Hospital and Cairo Specialized Hospital in Cairo city, Egypt during a period of 24 months (from September 2011 to August 2013). Patient's specimens were taken from urinary tract infection cultured on solid media. The identification of all clinical bacterial isolates was performed using API strips as described by the manufacturer (bio Merieux® Vitek Systems, France) as described before in details by **El-Sheikh *et al.* (2016)**.

Medicinal plants:-

The fifteen medicinal plants used in this study were summarized in table (1).

Fresh leaves and aerial parts of plants were washed by distilled water and dried by air at room temperature away from sunlight for six days. The dried medicinal plant materials were crushed into powder using grinding machine (Siemens-blender). They were then stored in a dry bags at room temperature a till extraction.

Medicinal Plant Extraction:-

Three different methods were used for extracting the active components from the 15medicinal plants used.

Hot Water Extraction:-

Aqueous extracts were prepared according to the method of **Li *et al.* (2006)** with slight modifications. Briefly, 50 g of the powdered plant material was mixed with 200 mL of distilled water in a conical flask, which was boiled, and shaken for 30 minutes in boiling water bath. The resulting mixture was allowed to cool to room temperature before being filtered using Whatman® No. 1 filter paper. Crude extracts were centrifuged at 4000 *xg* for 15 min. The water extracts were concentrated by heating in a water bath then filtered again using 0.45 µm aqua membrane nylon filter (Becton Dickinson® Company) to obtain the sterile extracts. The concentrated extracts were kept in sterile glass bottle at -20^{o}C.

Ethanol Extraction:-

Plant materials were extracted by ethanol following the method of **Goze *et al*. (2009)**, with slight modifications. Briefly, 50 g of the powdered plant samples were soaked in 200 ml of the 80% ethanol for three days, after which the extracts were filtered through Whatman® No.1 filter paper. Solvent was evaporated with a rotary evaporator (Buchi® Rotavapor R-124, Switzerland) and then filtered again using a 0.45µm membrane nylon filter (Becton Dickinson® Company) to obtain the sterile extracts.

Extraction of Essential Oils Using Steam Distillation method:-

Essential oils were isolated from all the plant materials by using Clevenger-type apparatus. Two hundred and fifty g from each plant were taken and placed into 2 L flask. Plant pieces were covered with 1.5 L of distilled water.Steam with essential oil vapors is condensed in thecondenser and is collected in a small round flat-bottom flask after 4-6 hours. The essential oil was separated using a reparatory funnel, dried underanhydrous sodium sulphate, transferred into a dark glass vials and stored at -20^{o}C until used **(A.O.A.C, 1995).**

Table 1:- The medicinal plants selected in this study and their used parts.

No.	Common Name	Botanical Name	Family	Used Part(s)
1.	Camel grass	*Cymbopogon proximus*	*Gramineae*	Leaves
2.	Celery	*Apium graveolens*	*Apiaceae*	Aerial parts
3.	Cinnamon	*Cinnamomum zeylanicum*	*Lauraceae*	Bark
4.	Clove	*Syzygium aromaticum*	*Myrtaceae*	Flower buds
5.	Dill	*Anethum graveolens*	*Apiaceae*	Aerial part
6.	Echinacea	*Echinaceae purpurea*	*Asteraceae*	Leaves
7.	Eucalyptus	*Eucalyptus globulus*	*Myrtaceae*	Leaves
8.	Fennel	*Foeniculum vulgare*	*Apiaceae*	Fruits
9.	Fenugreek	*Trigonella foenumgraecum*	*Fabaceae*	Seeds
10.	Ginger	*Zingiber officinale*	*Zingiberaceae*	Rhizomes
11.	Marjoram	*Origanum majorana*	*Lamiaceae*	Aerial part
12.	Parsley	*Petroselenium sativum*	*Umbellifereae*	Aerial part
13.	Rosemary	*Rosmarinus officinalis*	*Lamiaceae*	Aerial part
14.	Sage	*Salvia officinalis*	*Lamiaceae*	Leaves
15.	Thyme	*Thymus vulgaris*	*Lamiaceae*	Aerial part

Photography of Camel grass (*Cymbopogon proximus*)

Photography of Celery (*Apium graveolens*).

Photography of Marjoram (*Origanum majorana*).

Photography of Cinnamon (*Cinnamomum zeylanicum*).

Photography of Clove *(Syzgium aromaticum).*

Photography of Rosemary (*Rosmarinus officinalis*).

 **Photography of Dill (*Anethum graveolens*).**	 **Photography of Echinacea (*Echinacea purpurea*).**	 **Photography of Thyme (*Thymus vulgaris*).**
 **Photography of Eucalyptus (*Eucalyptus globulus*).**	 **Photography of Fennel (*Foeniculum vulgare*).**	 **Photography of Parsley (*Petroselinum crispum*).**
 **Photography of Fenugreek (*Trigonella foenum graecum*).**	 **Photography of Ginger *(Zingiber officinale).***	 **Photography of Sage (*Salvia officinalis*).**

Fig.1:- Photography of medicinal plants selected in this study and their used parts.

Antibacterial Activity of Medicinal Plant Extracts:-

The antibacterial activity was carried out by agar disc diffusion assay and broth dilution assay. Dried extract was re-dissolved in the smallest possible volume of water or 20% Dimethyl sulfoxide (DMSO) to give stock solutions of high concentrations. Concentrations of aqueous and ethanolic extracts were recorded on a weight by volume (w/v) basis, while essential oil concentrations by volume/volume (v/v).

Disc Diffusion Assay:-

The Mueller-Hinton plates were inoculated with the bacterial suspension using a sterile swab to achieve a lawn growth. Sterile paper disks (6mm in diameter) (Whatman® filter paper No.1) were impregnated with 20µl of medicinal plant extract solution in different concentrations (12.5%, 25%, 50%, 75%, and 100%) then placed on the inoculated agar surface. All plates were sealed with sterile laboratory bags to avoid evaporation of the test samples. Plates were allowed to stand at room temperature for 60 min. to let the test plant materials diffuse into the agar, and afterwards, they were incubated at 37^{o}C for 24 hours. Results are determined by measuring the clear zone of inhibition **(Hewitt** and **Vincent, 2003).** Studies were performed in triplicate and mean value was calculated.

Broth Dilution Assay:-

The antimicrobial effects of the extracts of the selected medicinal plants against different MDRB were determined by the broth dilution method as described by **Tepe *et al.* (2004**). Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. To determine the MIC, two-fold Serial dilutions were prepared for each extract with sterile Mueller-Hinton broth. 0.5 ml of each bacterial suspension (1×10^{6} CFU/ml) was inoculated in tubes with different concentrations of the extracts. Two controls were prepared, one containing MH broth and bacterial suspension serve as bacterial control, and one containing plant extracts in MH broth serve as negative control. Un-inoculated broth serve as blank, used to calibrate the spectrophotometer. The tubes were incubated at 37^{o}C for 24 h. Inhibition of bacterial growth was determined by measuring the absorbance at 600 nm. The measurements taken before and after incubation were compared and a difference of less than 0.05 indicates no microbial growth. The lowest concentration that had no microbial growth was determined to be the MIC.

TEM observations of treated bacterial cells:-
Bacterial cells of both treated and untreated bacterial cells were observed under Transmissions Electron Microscope (TEM). The samples were prepared by standard protocol **(Croft, 1999).** Samples was fixed in 1% Glutaraldehde than washed in 0.1 M buffer, 1% Osmium tetraoxide was used for post-fixations and again washed with 0.1 M buffer. The samples were dehydrated in acetone, infiltrated and embedded in epoxy resin. Finally, the grids were dried in a desiccator and examined using TEM (JEOL 1010 Japan), for study biocidal action of essential oil and any morphological changes.

Chromatographic analysis of essential oils composition:-
Analysis of Essential Oil was done using GCMS to recognize the chemical composition of Thyme, Cinnamon, and Marjoram essential oils. The HP 5890 series II Gas Chromatograph interfaced to a 5973 Mass Selective Detector and controlled by HP Chemstation software (version b.02.05) was used. The chromatographic separation was achieved using a HP5-MS capillary column (30.0 cm x 25 mm x 0.25 mm). The column stationary phase comprised of 5:95% diphenyl: dimethylpolysiloxane blend. The operating GC condition was an initial oven temperature of 35^{o}C for 3 min, then programmed to 280^{o}C at the rate of 10^{o}C/min, and then kept constant at 280^{o}C (25 min). The injector and detector temperatures were set at 270^{o}C and the carrier gas was helium flowing at a rate of 1.2 ml/min. The mass spectrometer was operated in the electron impact mode at 70 eV and the mass range from 40 to 800 amu. Ion source and transfer line temperature was kept at 300^{o}C. Identification of the constituents was done on the basis of retention index, library mass search database (NIST and WILEY) and by comparing with the mass spectral data **(A.O.AC, 2005; Jérôme *et al*., 2014).**

Results and Discussion:-

In the present report, among one thousand and six hundred urine samples screened; only 65% showed bacterial infection. However, 919 samples (88.4%) were sensitive to the tested antibiotics; 121 samples (11.6%) recognized as multi-drug resistant bacteria. These bacterial isolates were identified and differentiated by cultural, morphological, and biochemical analysis. The results also indicated that the most of urinary tract infection diseases were by Gram negative bacteria (102 isolates; 84.3 %). *Escherichia coli* was the most predominant organism causing UTI in this study (Fig. 2) that represented by 58 isolates (47.9%), followed by *Klebsiella pneumonia* (21.5%), *Pseudomonas aeruginosa* (14.9%), *Staphylococcus aureus* (12.4%), *Enterococcus faecalis* (3.3 %).

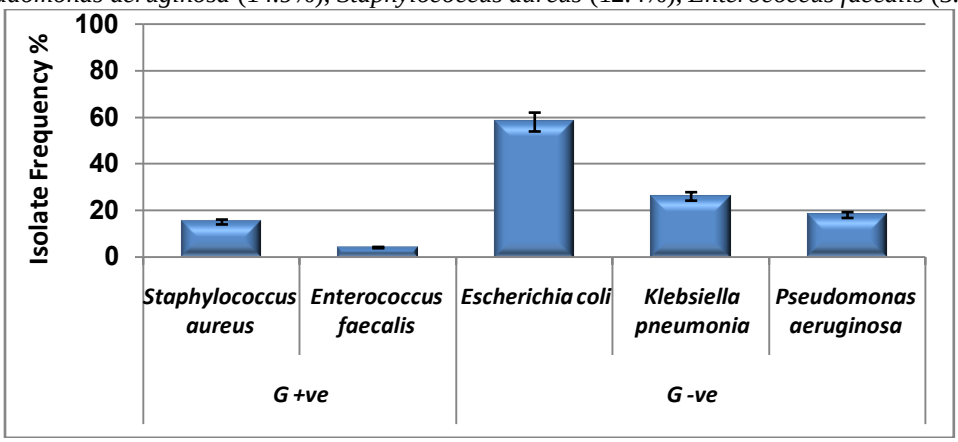


Fig. 2:- The percentage of the multi-drug resistant UTI bacterial isolates and differentiation using Gram reaction.

In this study, antibacterial activities of the 15 medicinal plants were tested on five selected MDR bacterial clinical isolates: *Escherichia coli, Klebsiella pneumoniae,Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus faecalis* that exhibited the highest resistance pattern.
In vitro antibacterial activity studies in the present study indicated that all plants essential oils and ethanolic or water extracts were found to be more effective at crude levels (100% concentration).
Out of 15 medicinal plants tested (Tables 2-16), cinnamon, thyme and marjoram showed maximum activity against all the bacterial isolates tested. Moderate effects were seen with clove, ginger, sage and rosemary. On the other hand, camel grass, celery, dill, echinacea, eucalyptus, fennel, fenugreek and parsley showed weak inhibition against tested clinical isolates.

High antibacterial activity was recorded for *Cinnamomum zeylanicum* compared to other plants with IZ values in range of 12.1 to 20.6 mm at 100% concentration. There was significant variation in the antibacterial activities (IZ values) of different plant extracts. The aqueous extracts of *Cymbopogon proximus* (Camel grass),*Apium graveolens* (Celery), *Syzygium aromaticum* (Clove),*Anethum graveolens* (Dill),*Echinaceae purpurea* (Echinacea),*Eucalyptusglobulus* (Eucalyptus), *Foeniculum vulgare* (Fennel),*Trigonella foenumgraecum* (Fenugreek), *Zingiber officinale* (Ginger),*Origanum majorana* (Marjoram), *Petroselenium sativum* (Parsley)and *Rosmarinus officinalis* (Rosemary) have shown weak antibacterial effect on isolated UTI pathogens.

Table 2:- Antibacterial activity of ***Petroselinum crispum*** "Parsley" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
Escherichia coli (370812)	-	-	-	8.6	9.4	-	-	9.8	11.4	12.1	-	8.3	13.0	13.5	15.4
Klebsiella pneumonia (410713)	-	-	-	-	8.4	-	-	-	9.3	11.4	-	-	8.3	11.6	12.5
Pseudomonas aeruginosa (270712)	-	-	-	-	-	-	-	-	8.8	10.3	-	-	9.5	10.7	12.3
Staphylococus aureus (100213)	-	-	8.4	10.4	12.4	-	8.6	10.2	11.1	14.6	-	10.0	11.3	14.5	16.3
Enterococcus faecalis (270412)	-	-	-	9.4	10.3	-	-	9.7	11.0	12.4	-	-	11.2	11.5	14.2

Table3:- Antibacterial activity of ***Apium graveolens*** "Celery" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	-	8.8	11.0	-	-	10.4	12.8	15.2	-	-	13.0	16.7	17.8
K.pneumonia (410713)	-	-	-	9.4	11.3	-	-	9.2	11.7	14.8	-	-	10.0	14.8	16.3
P.aeruginosa (270712)	-	-	9.4	11.1	13.1	-	-	10.1	13.3	16.3	-	7.8	10.8	13.0	15.7
S. aureus (100213)	-	-	10.0	12.7	14.3	-	9.1	14.3	15.7	17.8	9.0	10.6	15.3	19.0	20.4
E. faecalis (270412)	-	-	8.7	10.0	11.3	-	-	8.6	11.0	13.2	-	9.7	12.0	15.0	16.5

Table 4:- Antibacterial activity of ***Anethum graveolens*** "Dill" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	9.7	13.3	16.2	-	-	13.8	15.4	17.3	-	12.3	15.6	18.4	20.8
K. pneumonia (410713)	-	-	10.2	11.3	13.4	-	-	11.0	13.1	15.6	-	-	10.6	14.0	16.4
P. aeruginosa (270712)	-	-	-	11.4	13.7	-	-	-	12.3	15.7	-	-	11.8	13.3	16.5
S. aureus (100213)	-	-	8.5	10.3	12.5	-	8.4	10.6	12.2	14.4	-	10.4	11.8	18.4	23.3
E. faecalis (270412)	-	-	8.4	10.5	11.8	-	-	10.2	12.7	14.7	-	10.7	13.3	15.2	17.4

Table 5:- Antibacterial activity of ***Foeniculum vulgare*** "Fennel" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	-	7.1	8.1	-	-	-	10.1	11.8	-	-	10.3	11.7	13.5
K. pneumonia (410713)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa (270712)	-	-	-	-	7.2	-	-	-	-	10.6	-	-	-	-	12.0
S. aureus (100213)	-	-	-	9.2	11.5	-	-	8.6	10.3	12.7	-	-	10.5	13.6	15.1
E. faecalis (270412)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6:- Antibacterial activity of ***Trigonella foenumgraecum*** "Fenugreek" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	-	10.4	12.0	-	-	7.7	11.7	15.1	-	-	-	11.3	14.2
K. pneumonia (410713)	-	-	-		7.3	-	-	-	10.3	11.1	-	-	-	9.1	11.1
P. aeruginosa (270712)	-	-	-	7.4	10.7	-	-	-	10.4	13.3	-	-	-	9.5	11.1
S. aureus (100213)	-	-	7.0	7.5	9.1	-	-	8.6	11.5	14.6	-	-	-	11.5	12.8
E. faecalis (270412)	-	-	-	8.5	11.5	-	-	-	10.4	13.5	-	-	-	9.6	11.7

Table7:- Antibacterial activity of ***Cymbopogon proximus*** "Camelgrass" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	-	-	9.5	-	-	-	12.2	13.7	-	-	10.0	16.5	19.5
K. pneumonia (410713)	-	-	-	-	8.1	-	-	-	11.1	12.3	-	-	8.4	11.5	14.7
P. aeruginosa (270712)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus (100213)	-	-	-	-	12.3	-	-	12.7	15.3	18.3	9.1	13.2	17.8	21.3	24.6
E. faecalis (270412)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 8:- Antibacterial activity of ***Echinacea purpurea*** "Echinacea" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	-	-	7.8	-	-	8.0	9.5	10.2	-	-	10.6	12.6	15.0
K. pneumonia (410713)	-	-	-	-	-	-	-	-	-	9.3	-	-	-	11.3	13.1
P. aeruginosa (270712)	-	-	-	-	-	-	-	-	7.6	9.8	-	-	8.6	10.2	12.2
S. aureus (100213)	-	-	-	-	8.7	-	-	9.3	10.5	12.6	-	-	10.6	14.3	16.3
E. faecalis (270412)	-	-	-	-	7.5	-	-	-	9.3	11.2	-	-	8.3	10.7	12.6

Table 9:- Antibacterial activity of ***Eucalyptus globulus*** "Eucalyptus" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	-	-	8.2	-	-	-	-	10.5	-	-	10.4	11.1	13.1
K. pneumonia (410713)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa (270712)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus (100213)	-	-	-	-	10.0	-	-	-	11.5	12.6	-	11.3	15.1	18.2	21.1
E. faecalis (270412)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 10:- Antibacterial activity of ***Zingiber officinalis***"Ginger" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	8.5	10.4	11.2	-	-	11.6	13.4	16.2	-	11.5	17.7	22.0	26.4
K. pneumonia (410713)	-	-	-	-	7.5	-	-	-	10.6	12.0	-	-	10.2	12.4	14.1
P. aeruginosa (270712)	-	-	7.7	8.5	12.1	-	-	9.6	11.6	15.8	-	10.2	16.5	23.8	27.6
S. aureus (100213)	-	-	-	10.2	13.0	-	8.3	10.2	13.1	15.0	-	10.1	12.2	17.5	20.5
E. faecalis (270412)	-	-	-	8.1	9.6	-	-	-	9.8	12.1	-	-	9.0	13.3	15.1

Table 11:- Antibacterial activity of ***Rosmarinus officinalis*** "Rosemary" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	7.7	9.2	11.7	-	-	8.7	12.1	14.6	9.3	12.2	15.2	20.4	25.6
K. pneumonia (410713)	-	-	-	7.8	8.7	-	-	8.8	11.0	12.0	-	10.2	11.6	12.8	15.7
P. aeruginosa (270712)	-	-	-	9.6	10.7	-	-	10.2	12.5	17.2	-	11.2	15.8	20.3	23.6
S. aureus (100213)	-	-	10.4	12.0	12.4	-	8.4	11.8	15.2	17.6	9.0	11.8	19.3	28.3	34.0
E. faecalis (270412)	-	-	-	8.5	9.7	-	-	9.1	11.2	15.4	-	10.1	13.0	15.4	23.2

Table 12:- Antibacterial activity of ***Salvia officinalis*** "Sage" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	9.8	11.4	14.5	-	-	-	-	11.5	-	-	10.5	13.5	16.0
K. pneumonia (410713)	-	-	7.7	10.8	11.5	-	-	-	-	10.1	-	-	8.2	12.8	15.2
P. aeruginosa (270712)	-	-	-	8.6	11.4	-	-	-	8.7	10.7	-	-	8.7	11.6	14.8
S. aureus (100213)	-	-	10.0	13.0	15.3	-	-	-	10.8	14.1	-	-	11.5	17.0	20.4
E. faecalis (270412)	-	-	10.7	12.0	15.5	-	-	-	9.4	13.4	-	10.7	14.2	17.7	23.4

Table 13:- Antibacterial activity of ***Syzygium aromaticum*** "Clove" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	8.3	10.1	11.5	14.1	8.8	10.4	14.6	15.4	18.4	9.1	13.8	18.2	22.6	26.7
K. pneumonia (410713)	-	-	-	9.1	9.5	-	-	9.7	10.1	13.7	-	9.6	11.4	13.5	16.4
P. aeruginosa (270712)	-	-	8.6	9.6	13.2	-	9.3	12.2	15.1	17.2	-	10.4	14.1	19.8	24.7
S. aureus (100213)	-	7.6	8.7	10.6	11.7	9.2	19.4	25.8	29.7	34.7	10.5	16.5	20.0	24.2	33.0
E. faecalis (270412)	-	-	8.1	9.1	10.7	-	9.3	11.2	14.1	19.3	-	9.8	13.1	15.1	16.9

Table 14:- Antibacterial activity of ***Origanum majorana*** "Marjoram" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	-	7.6	9.0	11.0	-	10.1	12.6	16.2	18.7	10.7	15.8	23.5	31.8	36.1
K. pneumonia (410713)	-	-	-	8.0	10.7	-	-	9.3	12.5	13.8	-	10.1	14.4	17.7	19.8
P. aeruginosa (270712)	-	-	-	9.8	12.5	-	9.5	10.7	11.8	15.4	9.1	12.0	16.0	21.3	27.2
S. aureus (100213)	-	-	8.8	10.5	11.1	9.6	13.1	17.4	19.6	24.2	11.4	16.8	27.3	36.4	40.3
E. faecalis (270412)	-	-	-	9.3	12.2	-	9.4	12.4	14.2	17.1	9.5	11.7	17.3	19.7	22.8

Table 15:- Antibacterial activity of ***Thymus vulgaris***"Thyme" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract / MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
E. coli (370812)	-	10.1	11.2	14.4	17.1	10.6	13.0	15.7	19.3	22.1	19.8	25.0	30.7	36.2	39.3
K. pneumonia (410713)	-	9.1	9.8	11.3	13.7	-	10.1	12.0	15.3	18.3	11.2	13.1	16.3	20.1	23.2
P. aeruginosa (270712)	-	7.8	10.2	11.0	12.5	11.8	15.5	18.5	22.5	25.7	17.6	20.8	24.8	28.8	33.1
S. aureus (100213)	-	10.4	12.4	14.3	15.8	14.8	18.2	23.2	28.2	32.3	21.3	27.7	34.4	42.1	47.2
E. faecalis (270412)	-	9.6	11.0	11.5	13.2	8.7	11.4	14.2	17.5	19.7	12.8	16.4	19.8	24.2	27.5

Table 16:-Antibacterial activity of ***Cinnamonum zeylanicum***"Cinnamon" by disc agar diffusion method expressed in inhibition zone diameter (mm).

Plant extract MDR isolates (Isolate code)	Hot water extract					Ethanol extract					Essential oil				
	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%	12.5%	25%	50%	75%	100%
***E. coli* (370812)**	-	9.8	13.1	16.7	20.6	12.1	16.1	19.1	24.0	26.8	17.8	23.0	30.1	34.7	42.1
***K. pneumonia* (410713)**	-	8.3	11.2	15.4	16.8	10.1	14.2	17.2	19.8	21.4	12.0	16.4	20.6	24.8	28.4
***P. aeruginosa* (270712)**	-	8.3	11.1	11.8	15.8	12.3	14.3	18.5	20.6	24.7	16.9	20.4	25.3	32.5	37.7
***S. aureus* (100213)**	-	10.7	14.0	16.5	18.3	14.5	16.7	20.4	22.8	26.1	20.8	25.0	35.4	39.6	46.3
***E. faecalis* (270412)**	-	7.5	8.4	11.1	12.1	9.2	11.0	15.1	19.0	22.2	15.0	17.8	24.3	29.4	32.6

Similar results had been previously reported by **Al-dhaher (2008).** The aqueous extracts of *Salvia officinalis* and *Thymus vulgaris* have shown moderate antibacterial activity. Results of **Alshwaikh *et al.* (2014)** also indicated the effect of parsley and celery, their stronger effects were against Gram-positive cocci followed by Gram-negative bacilli while their effect on *E. coli* was much less. Parsley and celery followed dill in their general effect. In another study of **Al-Kareemi (2012)** showed that ethanolic extracts from the parsley inhibited the growth of various species of Gram-positive and Gram-negative bacteria.

However, aqueous and organic extracts of dill seeds have exhibited potent antibacterial activity **(Kaur** and **Arora, 2009).**In the present study negligible inhibitory activity with aqueous extract was observed in some of the plants which may be due to loss of some active compounds during extraction process of the sample or there may be lack of solubility of active constituents in aqueous solution (**Sampathkumar *et al.*, 2008).**

In addition, the type of solvent used to extract herbs and spices appeared to have a major impact on their antimicrobial activity. This is probably due to the fact that, although the solvents were removed from extracts by evaporation, and most of the components with antimicrobial properties are aromatic or saturated organic compounds which are generally more soluble in solvents such as ethanol or methanol **(Dupont *et al.*, 2006; Weerakkody *et al.*, 2010; Witkowska *et al.*, 2013).**

The ethanolic extracts of all the plants have shown good antibacterial effect against the UTI isolates. The most effective antibacterial activity was recorded for *Cinnamomum zeylanicum* (Table 16) withmaximum effect observed against *Escherichia coli* (IZ value 26.8 mm) and least against *Klebsiella pneumonia* (IZ value 21.4 mm). This effect is in agreement with other researchers regarding the antibacterial effect against *Escherichia coli*; however there is a difference in the concentration of extract of cinnamon used in this study (**Yuste** and **Fung, 2006**).

In the present study, the alcoholic extracts of clove, ginger and thyme were the most effective than the aqueous extracts against *Escherichia coli* and *Staphylococcus aureus* isolates. These results are in agreement with that obtained by many authors (**Ayoola *et al.*, 2008; Al-Jiffri *et al.*, 2011; Fuad *et al.*, 2012).**

In particular, marjoram, clove, thyme and cinammon alcoholic extracts had broad spectrum antimicrobial activities especially against Gram-positive and Gram-negative bacteria. Several previous studies **(Braga *et al.*, 2007; Bayoub *et al.*, 2010)** have reported broad spectrum activities for these extracts due to the phenolic compounds (mainly, eugenol, carvacrol and thymol).
El-Kamali and **El-Karim (2009)** in their study on *Trigonella foenum-graecum* seeds indicated pronounced antibacterial activity of ethanol seed extract. Similar to the earlier findings in fenugreek seed, the findings of the present study also show significant antibacterial activity in a polar solvent like ethanol.

The results obtained in this study corroborate with those of **Akintobi *et al.*, (2013)** showed that the ethanol extracts of *Zingiber officinale* had a higher inhibitory activity against the test organisms than that of the water extracts. In our study, it was also observed that the ginger extractexhibited maximum inhibitory effect against *P. aeruginosa* and moderate antimicrobial activity against *S. aureus* similar to those reported by **Melvin *et al.* (2009).**

Similarly, in the present study, highest antibacterial activity was exhibited by essential oils plant extracts. Among all plant extracts highest IZ values were recorded for *Cinnamomum zeylanicum* in the range of 46.3 and 28.4mm against UTI bacterial isolates in following order *Staphylococcus aureus > Escherichia coli >Pseudomonas aeruginosa >Enterococcus faecalis >Klebsiella pneumoniae*. The next higher activity was observed for *Thymus vulgaris, Origanum majorana, Syzygium aromaticum, Zingiber officinale, Salvia officinalis* and *Rosmarinus officinalis*. The order of inhibition followed same pattern exhibited by *Cinnamomum zeylanicum.*

Also, the antibacterial effect of *Cinnamomum sp.* bark extracts was studied by where the highest activity was recorded against *Pseudomonas aeruginosa* of UTI origin (**Prabuseenivasan *et al*. 2006; Tabassum *et al.*, 2013)**; *Staphylococcus aureus*(**Syed, 2010).** However, **Chao *et al*. (2000)** reported cinnamon bark essential oil being the most effective on bacterial growth while marjoram was less effective. On the other hand, clove essential oil exhibited a broad spectrum antimicrobial activity **(Ayoola *et al.*, 2008; Gupta *et al.*, 2008).**

Thymus vulgaris essential oil belongs to essential oils with the most pronounced antimicrobial activity **(Ghaly, 2006; Iten *et al.*, 2009).** Investigations on phytochemistry of *Origanum majorana* essential oils originating from different area in the world and their antimicrobial activity were previously reported **(Jirovetz *et al.*, 2008; Roby *et al.*, 2013).** Sage extracts and essential oils were also reported to exhibit significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*(**Yasar *et al.*, 2005).**

In our study, rosemary essential oil was exhibited intermediate action against *Escherichia coli* compared with those previous reported by **Angioni *et al.* (2004) and Al-Jiffri *et al.* (2011).**

Fennel extracts were previously reported to be effective against all types of bacteria but the effect is more pronounced against Gram-positive bacteria **(Mohsenzadeh, 2007; Saviuc *et al.*, 2012).** However, **Saviuc *et al.*(2012)** reported antibacterial potential of fennel essential oil against *Pseudomonas aeruginosa* which was not observed in the present investigation.

The essential oil extracted from dill seeds show no inhibitory effect on the growth of *Staphylococcus aureus*. The inefficiency of dill essential oil against *Staphylococcus aureus* has been reported occasionally in the past **(Abed, 2007)** these results being in contrast with other studies that report strong antibacterial activity **(Singh *et al.*, 2005).**

This study also revealed that cinnamon essential oil showed the highest activity with MIC values ranging from 0.04 to 0.16mg/ml followed by thyme and marjoram essential oils with MIC values ranging from 0.625 to 2.5mg/ml (Table 17).

Table 17:- The minimum inhibitory concentration (mg/ml) of the cinnamon, thyme and marjoram extracts against the tested MDR bacterial isolates.

Plant extract	***Cinnamonum zeylanicum***			***Thymus vulgaris***			***Origanum majorana***		
MDR Uropathogen isolates	**Hot water extract**	**Ethanol extract**	**Essential oil**	**Hot water extract**	**Ethanol extract**	**Essential oil**	**Hot water extract**	**Ethanol extract**	**Essential oil**
***Escherichia coli* (370812)**	**2.5**	**0.312**	**0.078**	**2.5**	**0.625**	**0.156**	**5.0**	**2.5**	**0.625**
***Klebsiella pneumonia* (410713)**	**2.5**	**0.625**	**0.156**	**5.0**	**1.25**	**0.625**	**>5.0**	**5.0**	**2.5**
***Pseudomonas aeruginosa* (270712)**	**2.5**	**0.312**	**0.078**	**5.0**	**0.625**	**0.156**	**>5.0**	**2.5**	**1.25**

***Staphylococcus aureus* (100213)**	**2.5**	**0.156**	**0.039**	**2.5**	**0.156**	**0.039**	**5.0**	**1.25**	**0.625**
***Enterococcus faecalis* (270412)**	**2.5**	**0.625**	**0.078**	**2.5**	**1.25**	**0.312**	**>5.0**	**2.5**	**1.25**

However,the estimated minimal inhibitory concentrations of *Cinnamomum zeylanicum* are ranged from 0.21 to 0.63µl/ml (v/v) (**Zainal-Abidin *et al.*, 2013),** 0.8 to 3.2 mg/ml **(Prabuseenivasan *et al.*, 2006).**

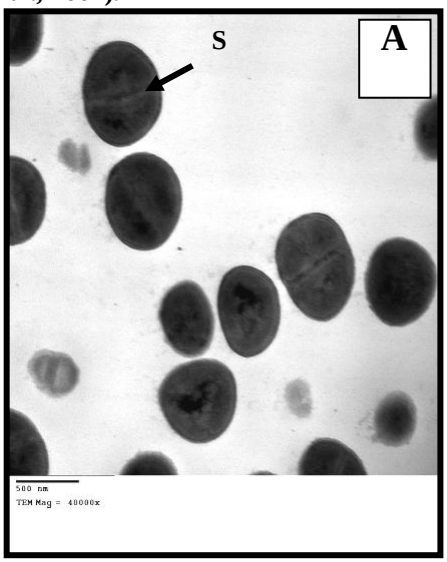
According to the antibacterial assay done for screening purpose, *Staphylococcus aureus* was the most susceptible Gram-positive bacteria to all plant extracts, whereas *Escherichia coli* was the most susceptible Gram-negative microorganisms. On the contrary, the Gram-negative MDR *Klebsiella pneumonia* was the most resistant microorganisms.

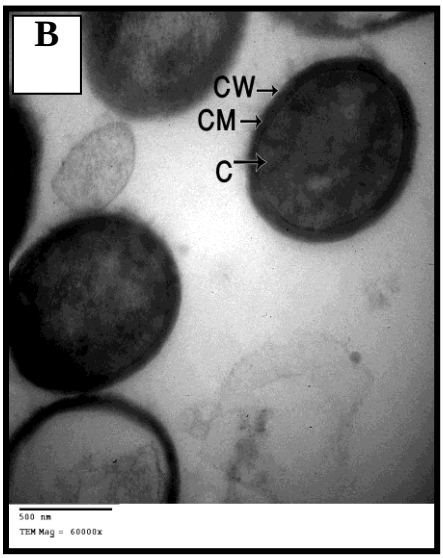
Effect of Cinnamon Essential Oil on Ultrastructure of Bacterial Cells:-
The effect of cinnamon essential oils on bacterial cell structure was tested using transmation electron microscopy on Gram-negative tested bacteria *Escherichia coli* and Gram-positive tested bacteria *Staphylococcus aureus.*

The untreated *Staphylococcus aureus* appeared cocci that displayed normally dividing cells with sharp delineation between cell wall, cytoplasmic membrane and the cytoplasm (Fig. 3A&B). After incubation of the bacterial cells with cinnamon essential oil (at 0.04 mg/ml), dramatic cellular alterations became visible on electron microscopic image (Fig. 3C&D). The treated cells appeared oblong; edges become abnormal, triangle, or elongated. Cell wall disrupted and exhibited thickened in some parts and breakdown in other due to leakage of cytoplasm.

Escherichia coli appeared short rods in TEM micrograph of untreated cells and showed a continuous thin smooth cell wall, cell membrane and nuclear material (Fig. 4A&B). When subjected of *Escherichia coli* cells to cinnamon essential oil at MIC: 0.08 mg/ml, bacterial cells lysed rapidly, incapable of septum formation, so cells appeared as very long threads (Fig. 4C). Cytoplasm shrinked leaving cell wall, while other cells appeared metamorphosed, cytoplasm lost its even distribution and showed clumping of intracellular materials (Fig. 4C&D). Cell wall was lost smoothness and uniformity and leading to cell wall rupture and even strong damage in many areas with thickened appearance more pronounced at polar-regions (Fig. 4C&D).

The current results indicating that, Gram-negative bacteria are generally more resistant to the antibacterial effect of than Gram-positive bacteria. However, **Kumar *et al.* (2012)** and **Upadhyay *et al.* (2010)** reported similar antibacterial potential against Gram-positive and Gram-negative isolates tested. While in other studies, Gram-positive bacteria were more resistant to the essential oils than Gram-negative bacteria **(Gupta *et al.*, 2008).** The better effectiveness of essential oils against Gram-positive bacteria than Gram-negative bacteria may be due to volatile action of essential oils and due to absence of lipo-polysaccharide layer in Gram-positive bacteria that might function as an effective barrier against any incoming biomolecule (**Delaquis *et al.*, 2002; Ming *et al.*, 2005; Shan *et al.*, 2007).**





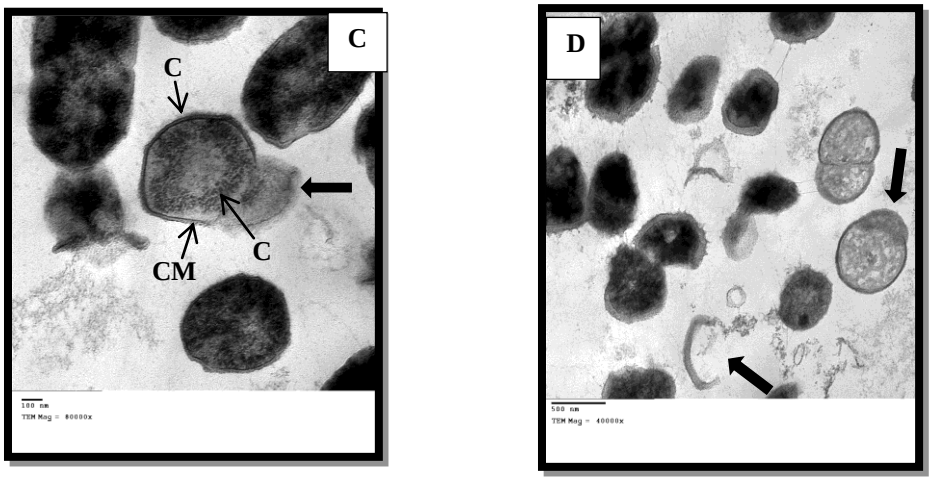


Fig. 3:- TEM microphotographs of *Staphylococcus aureus*. **A&B**: without treatment. **C&D**: treated with 0.04 mg/ml cinnamon oil. Where, S: septum, CW: cell wall, CM: cell membrane, C: cytoplasm. (A) x40000, Bar 500 nm (B) x60000, Bar, 500nm; (C) x80000 Bar 100 nm, (D) x40000, Bar 500 nm).

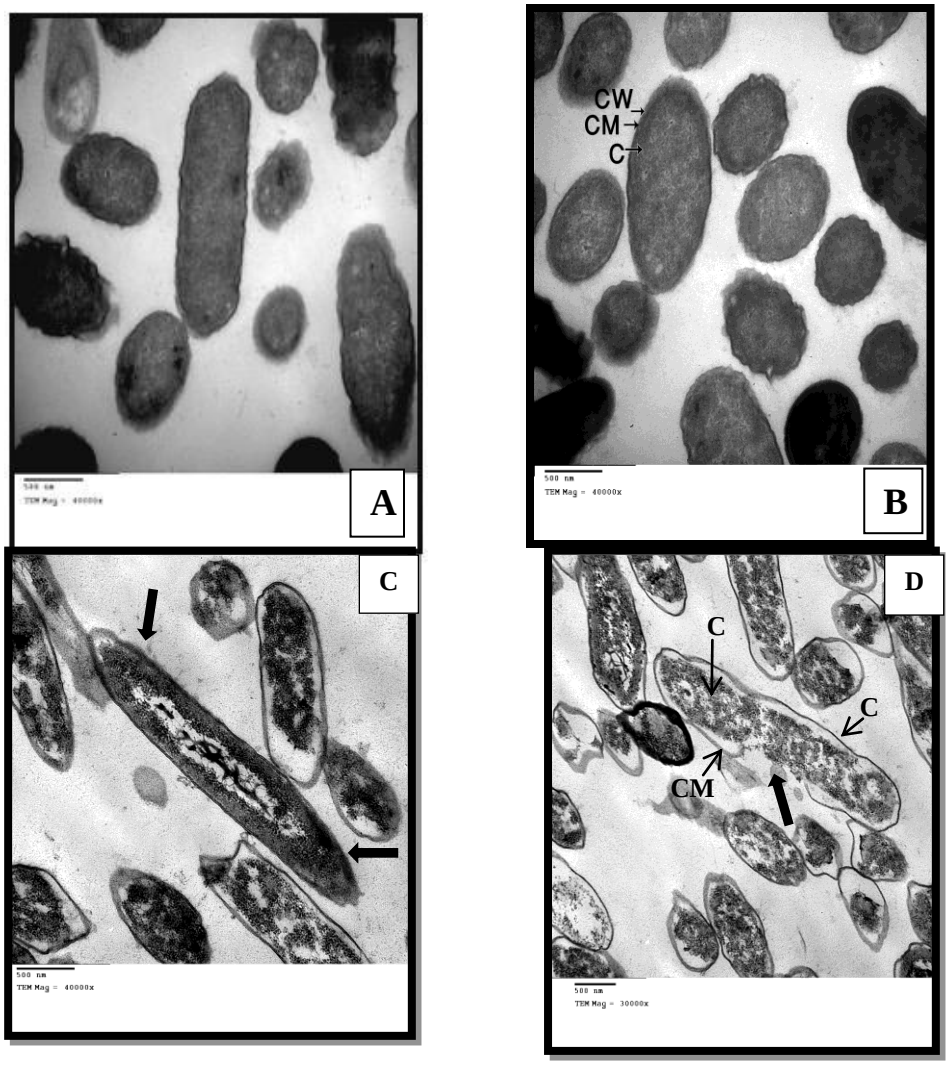


Fig. 4:- TEM microphotographs of ***Escherichia coli***. (A&B) cells without treatment, (C&D) treated with 0.08mg/ml Cinnamon oil. (A-C) x40000, Bar 500nm, (D) x30000, Bar 500nm. (CW: cell wall, CM: cell membrane, C: cytoplasm).

Moreover, in the current study GC/MS Analysis of essential oils indicated that marjoram oil had nearly sixteen major components (Table 18; Fig. 5). The main components were: linalool (29.20%) trpenin-4-ol (20.04%) and γ-terpinen (11.89%). Cinnamon oils had fifteen components (Table 19; Fig. 6), and the major ones cinnamaldehyde (72.87%) and cinnamic acid (8.88%). However, thyme oil had 17 major components (Table 20; Fig. 7) includes P-cymen (29.15%), thymol (24.80%) and carvecol (22.69%).

Table 18:- Chemical composition of Marjoram essential oil by using GC-MS.

Chemical Constituents	Peak No.	R.t	Area %
1- Cyclic terpenes:			
α -pinene	2	3.968	0.635
β -pinene	5	5.001	7.298
Limonene	7	6.445	6.86
α-terpinene	9	7.072	1.602
γ-terpinene	10	8.045	11.89
α-terpinolene	12	9.821	2.50
Total:			**30.785**
2- Aliphatic hydrocarbons:			
Myrcene	6	6.040	2.04
Total:			**2.04**
3- Aromatic hydrocarbons:			
P-cymene	11	8.715	0.561
1,8-cineol	8	6.830	1.96
Total:			**2.521**
4- Sesquiterpene hydrocarbons:			
Caryophellene	18	22.104	0.77
Total:			**0.77**
5-Terpine Ester:			
Linalyl acetate	15	19.599	2.17
Total:			**2.17**
6- Alphatic terpine alcohol:			
Linalool	14	19.286	29.20
Trans-sabinene hydrate	13	15.889	4.167
Total:			**33.367**
7- Cyclic terpine alcohol:			
Terpine-4-ol	17	21.188	20.04
α-terpineol	21	24.744	3.16
Borneol	22	25.400	1.14
Total:			**24.34**
Total known:			**96.00**
Total unknown:			**4.00**

Rt: Retention time calculated by minutes.

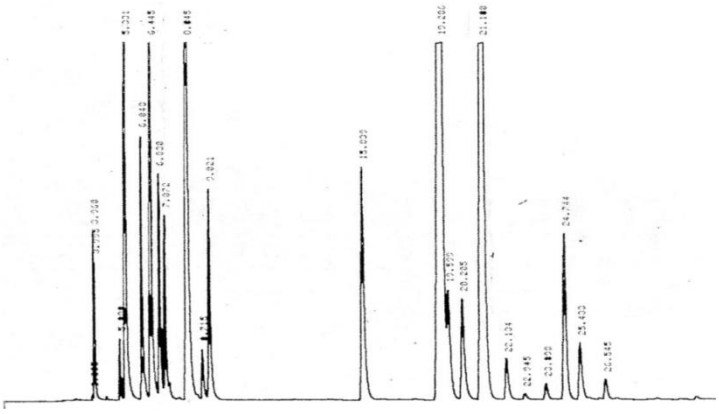


Fig. 5:- Gas chromatography (GC-MS) chromatogram showing chemical components of Marjoram essential oil.

Table 19:- Chemical composition of thyme essential oil by using GC-MS.

Chemical Constituents	**Peak No.**	**R.t**	**Area %**
1- Cyclic terpenes:			
α -pinene	5	4.124	0.71
β -pinene	7	5.283	0.41
Limonene	12	7.104	0.24
4-curene	8	5.65	0.09
α -phellandrene	11	6.685	1.63
γ -terpinene	14	8.407	7.09
Total:			**10.17**
2- Alphatic hydrocarbons:			
Myrcene	10	6.277	1.02
Trycline	4	3.904	0.04
Total:			**1.06**
3- Aromatic hydrocarbons:			
P-cymene	15	9.222	29.15
1,8-cineol	13	7.403	0.62
Total:			**29.77**
4- Sesquiterpene hydrocarbons:			
Caryophellene	25	20.843	1.47
Humulene	32	24.660	0.78
Total:			**2.25**
5- Alphatic terpine alcohol:			
Linalool	23	18.932	0.88
Total:			**0.88**
6- Cyclic terpine alcohol:			
Terpine-4-ol	26	20.967	2.35
Borneol	34	25.899	0.32
Thymol	45	42.691	24.80
Carvacrol	44	41.781	22.69
Total:			**50.16**
Total known:			**94.29**
Total unknown:			**5.71**

Rt: Retention time calculated by minutes.

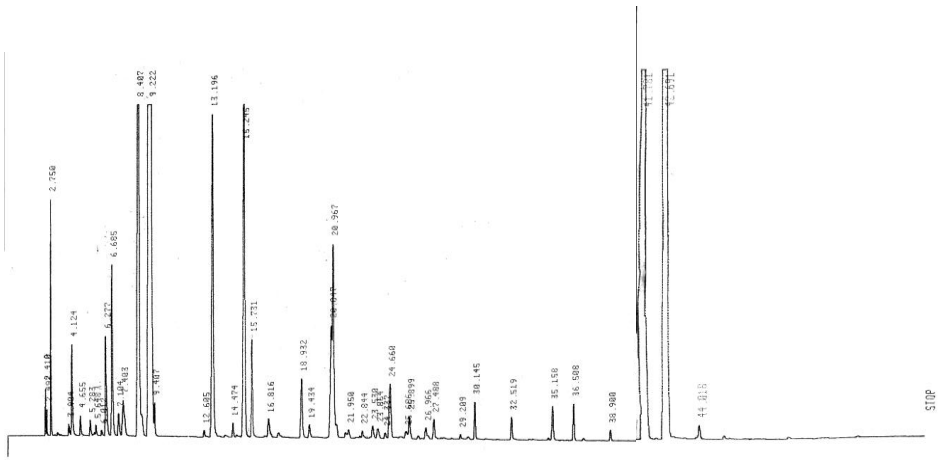


Fig. 6:-Gas chromatography (GC-MS) chromatogram showing chemical components of thyme essential oil.

Table 20:- Chemical composition of cinnamon essential oil by using GC-MS.

Chemical Constituents	Peak No.	R.t	Area %
1- cyclic terpines:			
α - pinene	2	4.040	0.64
β-pinene	4	5.345	0.20
Limonene	8	7.577	1.00
α -terpinene	18	25.121	0.34
Total:			**2.18**
2- Aliphatic hydrocarbons:			
β-myrcene	6	6.505	0.45
Total:			**0.45**
3- Aromatic hydrocarbons:			
P-cymene	11	9.32	1.033
Total:			**1.033**
4- Aromatic Aldehydes:			
Benzaldehyde	12	17.291	0.33
Cinnamic aldehyde	28	37.76	72.87
Total:			**73.20**
5- Aromatic terpine alcohol:			
Phenyl ethyl alcohol	2.40	32.79	0.35
Eugenol	32	41.58	1.72
Total:			**2.07**
6- Terpine Ester:			
Cinnamyle acetate	31	41.255	2.83
Total:			**2.83**
7- Alphatic terpine alcohol:			
Linalool	14	14.446	1.61
Total:			**1.61**
8- Sesquiterpene hydrocarbons:			
β-caryophellene	15	21.395	1.44
Camphene	3	4.655	0.24
Total:			**1.68**
9- Aromatic acid:			
Cinnamic acid	36	50.280	8.88

Rt: Retention time calculated by minutes.

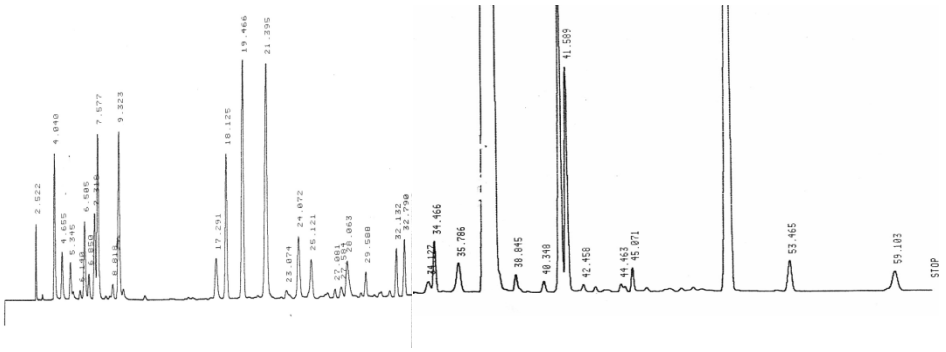


Fig. 7:- Gas chromatography (GC-MS) showing the chemical components of cinnamon essential oil.

The cinnamaldehyde being the major component of cinnamon bark oil is in agreement with those previously reported**.** Several studies have shown that cinnamon essential oil was very complex mixtures of compounds and many variations have been found in the chemical composition **(Wang *et al.*, 2009).** The antibacterial activity

ofcinnamon was probably due to their major component,cinnamaldehyde and their constituents is also known to inhibitsbacterial acetyl-CoA carboxylase and responsible for majorantibacterial activity **(Jantan *et al.*, 2008; Muthuswamy *et al.*, 2008; Meades *et al.*, 2011).**

Marjoram volatile oil is rich in terpinen-4-ol, sabinene hydrate, γ-terpinene, p-cymene, α-terpinene, and α-terpineol **(Lis *et al.*, 2007; Baatour *et al.*, 2012; Valeriano *et al.*, 2012).** Indicating that Egyptian marjoram oil belonged to terpinen-4-ol/sabinene hydrate chemotype as previously reported 25.09% **(Ayoola *et al.*, 2008),** 30.41% **(Busatta *et al.*, 2008),** 21.33% **(Jirovetz *et al.*, 2008),** 12.64% **(Badee *et al.*, 2013).** On the other hand, the main compounds identified for marjoram essential oil by **Freire *et al.* (2011)** were 4-terpineol (34.23%) followed by γ-terpinene (14.28%)**.**

However, these results confirmed the findings achieved by other authers **(Riad 2005; Busatta *et al.*, 2008),** who reported that marjoram essential oil possesses antimicrobial properties against pathogenic and spoilage microorganisms when tested in vitro.

High antimicrobial activity of thyme essential oil and its components, especially thymol and carvacrol, was demonstrated against *S. aureus* **(Soković *et al.*, 2010; Al-Bayati, 2008),** including methicillin-resistant isolates **(Tohidpour *et al.*, 2010)**, *E. faecalis* and *E. coli* **(Lević *et al.*, 2011),***P. aeruginosa***(Soković *et al.*, 2010),** and other microorganisms. Indeed, **Oussalah *et al.* (2006)** studied the antimicrobial properties of essential oils of several thyme species and found that despite a common botanical origin, the chemical composition and antimicrobial activity varied considerably.

According to the World Health Organization, thymol residues in food are without danger to the consumer as long as they do not exceed 50 mg/kg. Thymol is considered by many national authorities as generally recognized as safe (GRAS) (**FAO/WHO, 2008**).

The ability of phenolic compounds to alter microbial cell permeability, thereby permitting the loss of macromolecules from the cell interior, could help explain some of the antimicrobial activity **(Bajpai *et al.*, 2008; La Storia *et al.*, 2011).**

In conclusion, policies on the control of antibiotic usage have to be enforced due to higher resistance. In addition, the results of antibacterial assay in the current study revealed that essential oils and ethanol extracts of plant exhibited broad spectrum activity against tested isolates as compared to aqueous extract. Also, cinnamon essential oil showed the highest activity mainly due to effect of cinnamaldehyde on cell wall**.**

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