J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 5 (2): 43 - 56, February, 2014

EVALUATION OF ANTIMICROBIAL ACTIVITY AND PHYTOCHMICAL ANALYSIS OF CITRUS LEMON, MANDARIN, AND ORANGE PEEL

Abdel –Salam, A.F and Fatma A. A. Mostafa
Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt

ABSTRACT

The present study was carried out to find out the antimicrobial activity of ethyl acetate , acetone ,ethanol and petroleum ether of lemon *(Citrus lemone L*) ,mandarin(*Citrus reticulata L*.) and orange(*Citrus sinensis L*.) peel at different concentrations (5,10,25,50 mg/ml) antimicrobial analysis were done using disc diffusion technique and tube dilution method against *E.coli* O_{157}*: H* $_7$ *Staphylococcus aureus Pseudomonas flourescens* , *Pseudomonas aeruginosa, Candida albicans* and the results compared with the standard antibiotics ,e.g penicillin ,chloamphincol , erythromycin and chlorotetracycline The results showed that, the extracts at different concentrations (25,50 mg/ml) of lemon ,mandarin and orange peel exhibited the maximum zones of inhibition against *candida albicans* by using disc diffusion technique. On the other hand density least of the bacterial growth was obtained by using tube dilution method at 10 mg/ml concentration of different extracts against each *E.coli* O_{157}:H_7 and *Staphylococcus aureus*.As showed *candida albicans* not sensitive for all tested antibiotics.
Keywords: pathogenic bacteria – lemon- mandarin-orange extracts

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. Antibiotics are those substances which are produced by microorganism that kills or prevents the growth of another microorganism.

According to world health organization (WHO) , any plant which contain substances that can be used for therapeutic purpose or which are precursor of chemopharmaceuticals semi synthetic new drugs in referred as medicinal (Salih and Abass,2003).Oranges ,mandarins ,lime, lemon and grape fruits are commonly grown varieties of citrus . Citrus fruits products are known to potent antimicrobial agents like bacteria, fungus (Mathur *et al*; 2011). antimicrobial activity of plants had been received attention many years ago as one the most effective mechanism for the control of microorganism (Saadi *et al*., 2003).Citrus extracts have been shown to have antimicrobial properties in several foodstuffs (Fernandez *et al* .,2005).The peel of citrus fruits is a rich source of flavanones and many polymethoxylated , flavones which are very rare in other plants (Ahmed *et al* .,2006).The antimicrobial abilities of essential oils ,among which citrus oils ,are also shown to be particularly interesting field for applications within the food ,cosmetic industries and antimicrobial (Caccioni,*et* al.,1998).Herbs and spices with antimicrobial activity have been widely used both tradionally and

commercially to increase the shelf life and safety of food (Dupont *et al,.*2006).The use of plant extracts and phytochemical, both with known antimicrobial properties ,can be of great significance in therapeutic treatments (Seenivasan *et.al*.,2006). Citrus flavonoids have a large spectrum of biological activity including antimicrobial, antifungal, antidiabetic ,anticancer and antiviral activities(Ortuno *et.al*.,2006).In plants ,they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses (Sohn *et.al*.,2004).Many naturally occurring compounds present in plants , herbs and spices have been shown to posses antimicrobial effect against food borne pathogens (Deans and Ritchie,1987). antimicrobial activity of Eos from citrus fruits and their individual components has been widely demonstrated against molds and yeasts (Viuda *et.al*.,2008).Regarding the inhibition and reduction in numbers of foodborne pathogens such as *Salmonella typhimurium , E.coli* O_{57}*:*H_7 *,Listeria monocytogenes* and *Staph.aureus* by citrus Eos.scarce reported have been carried out (Fisher *et.al*.,2007).The ethyl acetate extract of citrus peel showed antibacterial activity against *Staph. aureus* and *E.coli (Chanthaphon et.al., 2008)* . The antibacterial activity of lemon peel against *Pseudomonas aeruginosa, Salmonella typhimurium* and *Micrococcus aureus* was also reported (Jai *et.al*.,2011).The antimicrobial activity of medicinal plant extracts have been linked to the presence of bioactive compounds which sometimes serve to protect the plants themselves against bacteria ,fungi and viral infections as well as exhibiting their antimicrobial properties on the microorganisms (El-Mahmood and Ameh ,2007).Active principles are found in various part of tangerine (mandarin)fruits such as the seed , peels and pulp, which include citric acid, coumarins , flavonoids and flavonones which have antibacterial and anti-inflammatory (Habib *et.al*.,1986).the ethyl acetate extract of citrus peel showed antibacterial activity against *Staph. aureus and E.coli (Chamthaphon et.al.2008),*the antibacterial activity of lemon peel against *Pseudomonas aeruginosa, Salmonella typhimurium* and *Micrococcus aureus* was also reported (Jai *et.al*.,2011). *Citrus Senensis* (sweet orange) has possesses anti-inflammatory, antibacterial and antioxidant properties (Ramachandran *et.al.,*2002).

The present study is carried out to evaluate the antimicrobial activity of different solvents extracts (ethyl acetate, acetone ethanol and petroleum ether) of lemon, mandarin and orange against bacteria(*E.coli* O_{57}*:*H_7 , *Staph. aureus* . *Pseudomonas aeruginosa, Pseudomonas flourescens*) and yeasts (*Candida albicans*) pathogens for antimicrobial activity

MATERIALS AND METHODS

Materials: Mandarin *(Citrus reticulata L.)*, orange *(Citrus sinensis L*.) And lemon *(Citrus lemone L.)* was purchased from the local Giza- Egypt market the fruits were washed under water tap and were peel. The peel dried by hot air flow (40 - 50°C) then stored in dark and cool place. Dried peels were ground before use and stored in dark at room temperature.

Solvents: Four solvents were used in this experiment ethyl acetate, ethanol, acetone and petroleum ether were purchased from Sigma company U.S.A.

Preparation of extract: The peel of different citrus fruits was prepared by soaking 200g of the material in 800ml various solvents separately (ethyl acetate, ethanol, acetone and petroleum ether) for 72 h and after every 24h. The mixture was stirred with a sterile glass rod. After the completion of 72 h time period the extracts were filtered with Whatmann filter paper no. 1 the solvents removed by rotary-vacuum evaporator, and removed completely by nitrogen evaporator to yield dry extracts, which were stored in sealed vials at 4 ^{o}C. Each extract were transferred to glass vials and kept at 4º C before use. The extracts were dissolved in dimethyl sulfoxide (DMSO) to produce different concentrations 5, 10, 25and 50 mg/ ml

Phytochemical analysis (Qualitative analysis): The peel extracts were subjected to preliminary phytochemical screening was done according to the methodologyof (Thenmozhi and Sivaraj 2011)

Test for alkoloids*:* 2 ml filtrate was mixed with 1% HCl and about 6 drops of Mayor's reagents. A Creamish or pale yellow precipitate indicated the presence of respective alkaloids.

Test for tannins: 1 ml of the extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatanins.

Test for reducing sugars*:* The residue was re-dissolved in water on the water bath. To 2ml of the solution, in the test tube was added, 1ml each of Fehling's solutions A and B. The mixture was shaken and heated in a water bath for 10min. The color obtained was recorded. A brick-red precipitate indicates reducing sugar

Test for terpenoids (Salkowski test):5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test): 5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Flavonoids: 0.2 ml of sample was added in 0.2ml of NaOH. And then add 1-2 drops of HCl, yellow to colorless showed the positive result

Microbial strain:

The microbial strains which used in this study e.g *E.coli* O_{57}*:*H_7 , *Staphylococcus aureus* , *Pseudomonas aeruginosa, Pseudomonas flourescens* and *Candida albicans* obtained from Dr Abdel –Salam, A.F Regional Center for Food and Feed, A. R. C., Giza, Egypt

Preparation of inculum:

Stock culture of *E.coli* $O_{57}H_7$, *Staph. aureus*, *Pseudomonas flourescens* and *Pseudomonas aeruginosa* were cultured in nutrient broth at 37 ^{O}c for 24 hr for *E.coli* $O_{57}:H_7$ and *Staph,aureus* and at 30^{O}c for 24hr for *Ps.flourescens* and *Ps. aeruginosa*. *Candida albicans* was cultured in Malt extract broth at 30^{O}c for 24hr.

The plant extracts (ethyl acetate, acetone ethanol and petroleum ether) were prepared from lemon ,mandarin and orange at different concentrations (5,10,25,50mg/ml) and antibiotics (penicillin 10µg,chloramphenicol 30 µg, erythromycin 15 µg and chlortetracycline30 µg), then tested for antimicrobial activity against *E.coli* $O_{57}:H_7$, *Staphylococcus ,aureus* , *Pseudomonas aeruginosa, Pseudomonas flourescens* and *Candida albicans*, using disk diffusion technique and tube dilution method .

Antimicrobial screening: disk diffusion: Petri dishes with nutrient agar medium were used for *E.coli* $O_{57}:H_7$,*Staph,aures*, *Ps.flourescens* and *Ps. aeruginosa* and Petri dishes with Malt extract agar medium were used for *Candida albicans* after inoculated it with microbial and yeast cultures.

The microorganisms and growth media were mixed through to ensure uniform distribution of the microorganisms the sterilized disks (6mm) were impregnated with 50µl of each dilution (5,10,25,50mg/ml) to each filter paper disk and placed on agar surface using forceps dipped in ethanol and flamed .antibiotics disks (penicillin ,chloamphincol , erythromycin and chlorotetracycline) were placed on the surface agar using forceps dipped in ethanol and flamed also .Plates for antimicrobial activity test were incubated at 37^{O}c for 24hr for *E.coli* $O_{57}:H_7$ and *Staph. aureus* and 30^{O}c for 24hr for *Ps.flourescens* , *Ps. aeruginosa* and *C.albicans* .The experiment was done three times .

The inhibition zone diameter was measured (including the filter paper disc 6mm in diameters) using Vernier calipers and express in millimeters(Konman *et.al.,*1997)

Tube dilution method:

Test tube each it contained 10ml of nutrient broth were inoculated with 0.2ml broth culture by either for *E.coli* $O_{57}:H_7$, *Staph,aureus*, *Ps.flourescens* and *Ps. aeruginosa* and another tubes each it contained 10ml Malt extract broth were inoculated with 0.2ml broth culture by *C.albicans, then* added 0.2ml of each different concentrations (5,10mg/ml) of plant extracts , (ethyl acetate, acetone, ethanol and petroleum ether) to the tube separately. The tubes for *E.coli* $O_{57}:H_7$ and *Staph,aureus* were incubated at 37^{O}c for 24 hr on rotary shaker (100 rpm), the tubes for *Ps.flourescens* , *Ps. aeruginosa* and *C.albicans* were incubated at 30^{O}c for 24 hr on rotary shaker(100rpm),after which turbidity reading was taken using Digital Direct-reading turbimeter.The controls were inoculated with only microbial strain or with adding dimethylsulfoxide(DMSO) with the same experimental condition as mentioned before.

RESULTS AND DISCUSSION

Inhibition zone (mm) using disc diffusion method:

The present results showed that the concentrations (5.10 mg/ml) of each ethyl acetate, acetone, ethanol and petroleum ether of lemon, mandarin and orange at 50 µl/disc were not sensitive for *E.coli* O_{127}*:* H_7*, Staph. aureus, Pseudomonas flourescens , Pseudomonas. aeruginosa* and *Candida albicans* therefore tested the concentrations (25 and 50 mg/ml) for the same solvents and microorganisms , with following the results recorded in Table (1) revealed that acetone extract of lemon at concentration 50 mg/ml was more the solvents effective for *E.coli* O_{157}*:* H_7 and *Ps.aeruginosa* such inhibited them with diameter inhibition zones 15 and 20 mm respectively. *Staph aureus* was more sensitive for ethyl acetate at concentration 50 mg/ml such exhibited inhibition zone 14 mm.

The concentrate 50 mg/ml of ethanol represent the optimum concentration of lemon extracts against *C. albican*s such exhibited diameter of inhibition zone 60 mm. on the other hand
Ps.flourescens showed similar sensitive for all solvents at different concentrations.

Table (1):Antimicrobial activity of lemon extracts against pathogenic bacteria (inhibition zone, mm)

Concentrations (mg/ml) / microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether	
	25	50	25	50	25	50	25	50
E.coli O_{157} *:*H_7	10	12	11	15	10	12	10	12
Staph aureus	12	14	7	9	7	10	7	9
Ps.flourescens	7	10	7	10	7	9	8	10
Ps.aeruginosa	10	12	12	20	13	15	11	12
C.albicans	40	50	40	50	45	60	10	13

Data recorded in Table (2) showed that *E.coli* O_{157}*:* H_7 was more sensitive for each acetone and ethanol extracts of mandarin at concentration 50 mg/ml such recorded inhibition zone 14 mm for each at. The ethanol extract at 50 mg/ml concentration exhibited higher diameter of inhibition zone against *Staph aureus* in 13 mm , while petroleum ether extract at concentration 50 mg/ml recorded higher inhibition zone in 25 mm against *Ps.aeruginosa*. on the other hand the zone of inhibition reached to 70 and 60 mm in diameter against *C.albicans* by ethyle acetate and ethanol extracts respectively. All mandarin extracts at different concentrations (25 and 50 mg/ml) induced similar effective against *Ps.flourescens*.

Table (2): Antimicrobial activity of mandarin extracts against pathogenic bacteria (inhibition zone, mm)

Concentrations (mg/ml) / microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether	
	25	50	25	50	25	50	25	50
E.coli O_{157} *:*H_7	10	12	12	14	12	14	11	13
Staph aureus	10	12	9	12	11	13	9	11
Ps.flourescens	7	10	7	10	7	10	7	10
Ps.aeruginosa	12	15	14	17	18	20	18	25
C.albicans	50	70	30	35	35	60	40	45

Form the summerized results recorded in Table (3) it is evident that *E.coli* O_{157}: H_7 was more sensitive toward petroleum petroleum ether extract of orange at 50 mg/ml concentration than another extracts , such showed inhibition zone in 15 mm diameter. Ethyle acetate and ethanol were had similar antimicrobial activity against *Staph aureus* especially at 50 mg/ml concentration, such inhibition zone reached to 12 mm in diameter for each it. Ethyle acetate was exhibited strong antimicrobial activity against *Ps.aeruginosa* in 35 mm diameter at 50 mg/ml concentration, while each ethyle acetate and acetone at 50 mg/ml concentration exhibited higher antimicrobial activity against *C. albicans* such that inhibition zone reached 55 mm in diameter for each it. Also *Ps. flourescens* revealed similar sensitive for all orange extracts at different concentrations.

Table (3) Antimicrobial activity of orange extracts against pathogenic bacteria (inhibition zone, mm)

Concentrations (mg/ml) / microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether	
	25	50	25	50	25	50	25	50
E.coli O_{157} :H_7	10	12	10	13	9	11	10	15
Staph aureus	10	12	9	11	8	12	8	10
Ps.flourescens	7	10	8	10	7	10	8	10
Ps.aeruginosa	15	35	17	30	12	25	18	20
C.albicans	35	55	30	55	25	45	20	40

Antimicrobial activity assay using tube dilution method:

The obtained results in Table(4) cleared showed that ethyle acetate extract of lemon at concentration 10 mg/ml induced decreasing of *Ps. aeruginosa* density form 85 to 19 nephlometric turbidity unit (NTU), while acetone at concentration 10 mg/ml resulted in logarithmic decrease of *Staph aureus* and *Ps. flourescens* density from 77 and 89 to 7 and 20 NTU respectively . Also petroleum ether induced logarithmic decrease of *C.albicans* density from 71 to 12 NTU. Similar each ethyle acetate and ethanol extract of lemon at concentration 10 mg/ ml in decrease of *E.coli* O_{157}: H_7density from 77 to 12 NTU.

Table (4) Antimicrobial activity assay of lemon extracts with tube dilution method by using nephelometric turbidity unit (NTU).

Concentrations (mg/ml) / microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether		Control A	Control B
	5	10	5	10	5	10	5	10		
E.coli O_{157} :H_7	24	12	23	17	20	12	33	17	89	80
Staph aureus	15	9	20	7	16	10	22	10	83	77
Ps.flourescens	39	22	37	20	38	31	45	27	96	89
Ps.aeruginosa	27	19	32	25	37	26	40	25	93	85
C.albicans	27	16	24	18	28	16	17	12	78	71

C.A:control with only microbial strain
C.B:control with microbial strain and DMSO

The results in Table (5) obviously showed that ethanol extract of mandarin at concentration 10 mg/ml resulted logarithmic decrease of *E.coli* O_{157}: H_7 density from 80 to 0.3 NTU. Each ethyle acetate and ethanol extract at concentration 10 mg/ml exhibited similar effective against *Staph aureus* such induced decease of *Staph aureus* density from 77 to 0.4 NTU.

Table(5):Antimicrobial activity assay of mandarin with tube dilutionmethod by using nephelometric unit (NTU).

Concentrations (mg/ml) / microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether		Control A	Control B
	5	10	5	10	5	10	5	10		
E.coli O_{157} :H_7	17	10	0.8	0.5	0.6	0.3	13	0.8	89	80
Staph aureus	16	0.4	0.8	0.6	0.6	0.4	16	0.8	83	77
Ps.flourescens	26	17	25	0.9	14	9	31	20	96	89
Ps.aeruginosa	24	6	19	12	13	10	22	16	93	85
C.albicans	23	16	11	7	14	10	14	0.6	78	71

The same footnotes in Table (4)

The concentration 10 mg/ml of acetone extract of mandarin resulted logarithmic decrease of *Ps.flourescens* density from 89 to 0.9 NTU , and ethyle acetate induced decreasing of *Ps.aeruginosa* density from 85 to 6 NTU at the same concentration. Also petroleum ether at concentration 10 mg/ml resulted logarithmic decrease of *C.albicans* from 71 to 0.6 NTU.

Data recorded in Table (6) clearly showed that ethanol extract of orange at concentration 10 mg/ml represented the optimum concentration for logarithmic decrease of each *E.coli* O_{157}:H_7, *Staph aureus* , *Ps.aeruginosa* and *C.albicans* density from 80,77,85 and 71 to 0.2, 0.3 ,0.5 and 0.9 NTU respectively , while acetone extract at concentration 10 mg/ml resulted logarithmic decrease of *Ps.flourescens* density from 89 to 7 NTU.

Table(6):Antimicrobial activity assay of orange extracts with tube dilution method using nephelometricturbidiry (NTU).

Concentrations (mg/ml) / microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether		Control A	Control B
	5	10	5	10	5	10	5	10		
E.coli O_{157} :H_7	11	5	0.9	0.6	0.5	0.2	12	0.6	89	80
Staph aureus	0.7	0.4	0.6	0.4	0.6	0.3	0.6	0.4	83	77
Ps.flourescens	30	18	13	7	15	8	20	16	96	89
Ps.aeruginosa	18	11	14	9	0.9	0.5	16	11	93	85
C.albicans	18	12	20	14	12	0.9	15	9	78	71

The same footnotes in Table (4)

The different studies recoded that the citrus peel oils showed strong antimicrobial activity against the Gram (+) and Gram (-) and the fungi cultures (Gulay *et al* ., 2009). The extracted orange oil could effectively inactivate *S.typhimurium* and *E.coli* but not *Staph aureus* (Lin, *et al*., 2010). The citrus peel oils of lemon showed strong antimicrobial activity against microorganisms like *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Micrococcus aureus* (Danavade, 2011). The zone of inhibition for citrus lemon against each *E.coli* and *Staph aureus* was 14 mm (Gulay, *et at* .,2009). The

highest antibacterial potentiality was exhibited by the ethyl acetate peel extract of citrus lemon against *Staph aureus , E.coli* and *Salmonella typhi* (Kumar *et al* ., 2011). Hydromethanolic extract of orange peel exhibited antibacterial against *Staph aureus* (6-14mm), *E.coli* (7-12mm) and *Pseudomonas. aeruginosa* (6-9mm) (Dubey *et a*l., 2011). The antibacterial effects of the ethonolic extracts suggest their possible use for the treatment of infections caused by *Salmonella paratyphi* (Lawal *et al* .,2013). The leaf and fruit peel of mandarin showed significant antimicrobial activities against most common Gram positive and Gram negative bacteria and some fungi (Hamdan ,*et al*.,2013) Many studies confirmed the antimicrobial activity of fruit peel oil of many citrus species. Including Cleopatra mandarin (Espina *et at*.,2010). The antimicrobial activity is related to ability of trepenes to effect not only permeability but also other functions of cell membranes, there compounds, might cross the cell membranes , thus penetrating into the interior of the cell and interacting with critical intracellular sites (Cristani *et al*.,2007). Ethanolic extract of lemon seeds showed maximum zone of inhibition against *Pseudomonas aeruginosa* whereas hot water extract showed least zone of inhibition (Pandey,*et al*.,2011). The zone of inhibition of tangerine seed ethanolic extract on *Staph aureus* was 20 mm*, E.coli* 15 mm and *klebsiella pneumonia* 12 mm , while the zones of inhibition shown by the petroleum extract were 15 mm for *Staph aureus*, 9mm for *Escherichia coli* and 7 mm for *klebsiella pneumonia*

(Agu *et al.*2013) Demonstrated the antimicrobial activity of tangerine peel oil obtained by steam distillation against *E,coli, Ps.aeruginosa,Salmonella paratyph* , *Staph aureus* ,proteus mirabilis and *Candida Albicams* (Ayoola *et al*., 2008).The antibacterial activity of tangerine oil (citrus reticulate blanco) variety Dancy at a concentration of >1% was bioactive against *Staph aureus* and *Literia monocytogenes* but had no activity against *E.coli* and *Ps.aereuginosa* (Martinez *et al* ., 2003).The crude ethanolic extract of senensis peel was found be active against the *Salmonella* species (lawal *etal*.,2013). Reported the antimicrobial activity of lemon peel against *Pseudomonas aeruginosa*,*S.typhimurium* and *Micrococcu saureus (Jai et al.,2011).*Extracts of both peels and leaves of citrus senesis showed moderate antimicrobial activity against *Staph aureus,Escherishia coli* and *Pseudomonas aeruginosa* (Omodamiro and Umekwe,2013).Orange oils have antimicrobial properties and may be applied in local therapies in the treatment of diseases caused by *Staph aureus*, *Ps.aeruginosa* ,*E.coli* and *Candida albicans*(Obidi *etal*,.2013) Citrus oils have antimicrobial properties not only against yeast ,moulds and spore forming bacterial but also food-poisoning bacteria (Deans and Ritchie,1987).Both *Ps.aeruginosa*,*E.coli,Staph aureus* and *C.albicans* were susceptible to undiluted lime juice (Rahman *et al*.,2010).
Data presented in Table (7) reveled that erythromycin 15µg was more sensitive than penicillin 10ug,chloramphenicol 30 µg and chlortetracycline 30 µg against *E.coli* O_{157}: H_7 and *Staph aureus* such exhibited diameter of inhibition zone 35 and 20 mm for *E.coli* O_{157}: H_7 and *Staph aureus* respectively. Penicellin 10µg showed similar antimicrobial activity against each *Ps.flourescens* and *Ps.aeruginosa* in 25mm for each it *Ps.flourescensand* was unsusceptible for chloramphenicol 30 µg and

erythromycin 15µg, and *Ps.aeruginosa* was not sensitive for chloramphenicol 30µg, erythromycin 15µg and chlortetracycline 30µg, but *C.albican*s was not sensitive for all tested antibiotics. Antimicrobial activity of ethyle acetate, acetone, ethanol and petroleum ether extracts of lemon, mandarin and orange were compared with some standard antibiotics as (pencillin , chloramphenicol, erythromycin and chlortetracycline)which use in treatment of patients against pathogenic bacteria followed by can be use appropriate antibiotics for those microbial isolates which used in this study and their substitute of natural antimicrobial agents.

Table (7):Antimicrobial activity of some standard antibiotics (µg) by inhibition zone diameter (mm).

Concentrations (mg/m) / microorganisms	Penicillin	Chloramphenicol	Erythromycin	chlortetracycline
	10	30	15	30
E.coli O_{157} :H_7	20	18	35	10
Staph aureus	15	16	20	10
Ps.flourescens	25	-	-	20
Ps.aeruginosa	25	-	-	-
C.albicans	-	-	-	-

The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al*., 2007). The screening phytochemcial investigation revealed the presence of various constituents of citrus peels. The results are shown in the Table (8). Different solvent showed different class of phytochemicals .they showed the presence of flavanods, terpenoids, glycosides etc. These constituents could account for the antibacterial activity but it is difficult to correlate their action to a specific phyochemical.The presence of phenol further indicated that citrus limon and citrus sinensis peels could act as antiinflammatory, anti clotting, antioxidant, immune enhancers and hormone modulators. Citrus peels can be used as a food preservative or even as food supplement as many literature says that they are highly nutritive

T8

CONCLUSION

Ethyl acetate, acetone, ethanol and petroleum ether extracts showed antimicrobial activity against *E.coliO_{157}: H_7, Staph aureus ,Ps.flourescens ,Ps.aeruginosa* and *C.albicans* .This study may thus lead to the formulation of an antimicrobial drug and can be used as a potent natural antioxidant additive or food products and as a dietary supplement.

REFERENCES

Agu, K.C.; Igweoha, C.A. and Umeh C.N. (2013). Antimicrobial Activity of the Ethanolic and Petroleum Ether Extracts of Tangerine Seed on Selected Bacteria International Journal of Agriculture and Biosciences 2(1): 22-24.

Ahmad, M.M.; Salim-ur-Rehman, Iqbal, Z.; Anjum F.M. and Sultan,. J.I. (2006).Genetic variability to essential oil composition in four citrus fruit species. Pak. J. Bot., 38(2): pp.319-324.

Akinmoladun, A.C.;Ibukun, E.O, Afor, E. Obuotor, E.M. and Farombi, E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Sci. Res. Essay, 2, pp.163-166.

Ayoola, G.A; Johnson, O.O. Adelowotan, T. Aibinu, I.E. Adenipekun, E. Adepoju-Bello, HAB Coker A.A. and Odugbemi, T.O. (2008). Evaluation of the chemical constituents and the antimicrobial activity of the volatile oil of Citrus reticulata fruit (tangerine fruit peel) from South West Nigeria. Afric J Biotechnol, 7: 2227-2231

Caccioni, D. R.; Guizzardi, M. Biondi, D. M. Renda, A. & Ruberto, G. (1998). Relationship between volatile components of citrus fruit essential oils and antimicrobial action on Penicillium digitatum and Penicillium italicum, International Journal of FoodMicrobiology, 43,pp. 73–79.

Chanthaphon, C.; Supitchaya, A. and Tipparat, H. (2008). "Antimicrobial activity of essential oil and crude extract from tropical Citrus sp against food-related micro-organisms", Songkhlanakarin J. Sci. Technol, vol. 30, no. 10, pp. 125-131,.

Cristani, M.; d'Arrigo, M. Mandalari, G. Castelli, F. Sarpietro, M. G. Micieli, D. (2007). Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. Journal of Agricultural and Food Chemistry,available on line

Espina ,l.S; Loreen , M. Conchello, S. Garcia,D. P. and Pageen, R. (2010).

Chemical compostion of commercial Citrus fruit essential oil and evaluation of their Antimicrobial activity acting alone or in combined processes . food control 22:896-902.

Danavade, M .J; Jalkute,C.B. Ghosh,J.S. and Sonawane ,K.D.(2011).Study Antimicrobial activity of lemon (Citrus lemon .L)peel extract .Br.J.pharmacol.Toxicol., 2 (3):119-122

Deans, S. G. and Ritchie, G. (1987). Antibacterial properties of plant essential oils. International Journal of Food Microbiology, 5(2), 165-180.

Dubey, D.;Balamurugan ,K. Agrawal ,R. Verma, R. and Jain,R. (2011). Evalution of antibacterial and antioxidant activity of methanolic and hydromethanolic extract of sweet orange peels .Rec.Rec.Sci.Tech 3(11) :22-25

Dupont, S.; Caffin, N.; Bhandari, B. and Dykes, G.A. (2006). In vitro antibacterial activity of Australian native herb extracts against food-related bacteria. Food Control, 17(11): 929-932.

El-Mahmood AM. and Ameh JM. (2007). In-vitro antibacterial activity of Parkia biglobosa (Jacq) root, bark extract against some microorganisms associated with urinary tract infections. Afric J Biotechnol, 6: 195-200.

Fernandez-Lopez J.; Zhi N.;Aleson-Carbonell L, Perez-Alvarez JA and Kuri V (2005). Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Sci., 69: 371-380.

Fisher, K., Rowe, C.and Phillips, C. A. (2007). The survival of three strains of Arcobacter butzleri in the presence of lemon, orange and bergamot essential oils and their components in vitro and on food. Letters in Applied Microbiology, 44(5), 495-499.

Gülay K.F.; Aydin T. Başaran, D.and Gülen T. (2009). Antimicrobial activity of Turkish citrus peel oils Pak. J.Bot., , 41(6), pp. 3207-3212.

Habib, M.A.; Hammam, M.A. Sakr, A.A. and Ashoush, Y.A. (1986). Chemical evaluation of egyptian citrus seeds as potential sources of vegetable oils J Amer Oil Chem Soci, 63: 1192-1197.

Hamdan, D. I.; Abdulla, R. H. Mohamed, M. E. and El-Shazly, A. M.(2013). Chemical composition and biological activity of essential oils of Cleopatra mandarin (Citrus reshni) cultivated in Egypt Journal of Pharmacognosy and Phytotherapy .5(5):83-90.

Jai, S. G.; Maruti, J. D. Chidamber, B. J. and Kailash, D.S. (2011). "Study of antimicrobial activity of Lemon (Citrus lemon L.) peel extract", British Journal of Pharmacology and Toxicology, vol. 2, no. 3, pp. 119-122.

Konman, Elmer W.; Allen, Stephen, D. Janda, William, M. Schreckenberger; Paul, C. and Winn, Washington, C. Jr (1997). Lippincott, color Atlas and textbook of diagnostic microbiology (5 th ed). New York; Philadelphia.

kumar ,K. A.; . Narayani, M. Subanthini, A. and Jayakumar, M. (2011). Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels Utilization of Fruit Waste International Journal of Engineering Science and Technology3(6):5414-5420.

Lawal, D.; Bala J. A. Aliyu, S. Y. and Huguma, M. A. (2013). Phytochemical Screening and In Vitro Anti-Bacterial Studies of the Ethanolic Extract of Citrus Senensis (Linn.)Peel against some Clinical Bacterial Isolates International Journal of Innovation and Applied Studies .2(2):138-145.

Lin ,C.M.;Sheu,S.R. Hsu,S.C. and Tsai,Y.H.(2010). Determination of bacterial efficacy of essential oils extracted from orange peel on the food contact surfaces . food control, (21) :1710-1715.

Martinez, J.; Sulbaran de Ferrer, B. Ojeda de Rodrı´guez, G. Ferrer, A. and Nava, R. (2003). Antibacterial activity of mandarin essential oil. Review of Faculty of Agronomy, 20: 502-512.

Mathur, .A.; Purohit, S. Gupta, R. Prasad, V. Mathur, D. Singh, S. and Singh, S. (2011). Evaluation of in vitro antimicrobial & antioxidant activity of peels & pulp of some citrus species. International journal of biotechnology & biotherapeutics,; (1)2 :2229-2278.

Obidi,O.F;Adelowotan,A.O. Ayool,G.A. Johnson,O.O. Hassann,M.O. and Nwachukwu,S.C. (2013) . Antimicrobial activity of orange oil on selected pathogen .the International Journal of Biotechnology ,2 (6):113-122.

Omodamiro, O. D.; and Umekwe, J. C.(2013). Evaluation of anti-inflammatory, antibacterial and antioxidant properties of ethanolic extracts of Citrus sinensis peel and leaves .Journal of Chemical and Pharmaceutical Research, 2013, 5(5):56-66.

Ortuno, A.A.; Baidez, P. Gomez, M.C. Arcas, I. Porras A.G. and. Del Rio, J.A. (2006). Citrus paradise and Citrus sinensis flavonoids: Their influence in the defence mechanism against Penicillium digitatum.Food Chem., 98(2): 351-358.

Pandey,A.; Kaushik A. and Tiwari, S. K.(2011). Evaluation of antimicrobial activity and phytochemical analysis of Citrus limon Journal of pharmaceutical and biomedical sciences 13 (17):1-7.

Rahman,S.; Parvez, A.K. Islam, R. and Khan, M.H. (2010). antimicrobial activity of natural spices on multi drug resistant esherichia coli isolated from drinking water Bangladesh Ann.Clin microbials antimicrobials10:10.

Ramachandran, S.; Anbu, J. Saravanan, M. Gnanasam, K. and Sridhar, S.K. (2002). Antioxidant and antiinflammatory properties of *Citrus sinensis* peel extract. Indian J. Pharm. Sci:., 64:66-68.

Saadi, M.A.K.; Salih, H.AL.M.and Abass. A.M (2003). Study of the fruit peels of Citrus sinesis & Punica granatum. journal of Babylon university (9) 3:243-342

Salih, H.AL.M. and Abass, A.M. (2003). Study of the fruit peels of Citrus sinesis & Punica granatum. journal of Babylon university. ;(9) 3:243-342.

Seenivasan, P., Manickkam J. and Savarimuthu, I. (2006). In vitro antibacterial activity of some plant essential oils. BMC Complem. Altern. M., 6: 39.

Sohn, H.Y.; Son, K.H. Know, C.S. and Kang, S.S (2004). Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: Morus alba L., Morus mongolica Schneider, Broussnetia papyrifera (L.) Vent, Sophora flavescens Ait and Echinosophora koreensis Nakai. Phytomedicine, 11:666-672.

Thenmozhi. M,and Sivaraj, R. R.H.A (2011). comparative Phytochemical of Alstonia scholaris ,Lawsonia inermis, Ervatamia divaricata and Asparagus racemosus.IJPRD,; 2(9):86-91.

Viuda-,M. M. Ruiz-,N. Y. Fernández L. J. and Pérez-Álvarez, J.(2008).Antifungal activity of lemon(Citrus lemon L.), mandarin (Citrus reticulata L.),grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils.Food Control19(12), 1130-1138.

تقيـيم النشـاط المضـاد للبكتريـا ووجـود المركبـات الفيتوكيمـائيـة فـى بعـض المستخلصـات لقشور الموالح الليمون والبرتقال واليوسفى

أحمد فريدعبد السلام و فاطمة أحمد على مصطفى
المركز الإقليمى للأغذية والأعلاف – مركز البحوث الزراعية – مصر

اجريت الدراسة لمعرفة النشاط المضاذ للبكتريا لمستخلصات , ethyl acetate, acetone ethanol , petroleum ether لقشورالليمون والبرتقال واليوسفى على التركزات (5و10و25و50مجم /مل)
تم عمل تحليلات النشاط المضاد للبكتريا باستخدام طريقة الأنتشار والأنابيب المخففة ضد *E.coli* O_{157} H_7: *Staphylococcus aureus* ,*pseudomonas flourescens* ,*pseudomonas aeruginosa, Candida albicans*
قورنت النتائج بتاثير المضادات الحيوية القياسية مثل البنسللين والكلورامفينكول والأريثروميسن والكلوروتتراسيكلين
اظهرت النتائج ان المستخلصات على التركزات المختلفة (25و50 مجم/مل) من قشور الليمون والبرتقال واليوسفى عرضت المساحات الأمثل من التثبيط ضد *Candida albicans*
باستخدام طريقة الأنتشار وعلى الجانب الأخر فان اقل كثافة من النمو البكترى قد حصل عليها باستخدام طريقة الأنابيب المخففة على تركيز (10مجم /مل) للتركزات المختلفة ضد كل من *E.coli* O_{157} H_7: *Staphylococcus aureus* وأوضحت النتائج ايضا ان *Candida albicans* كانت غير حساسة لكل المضادات الحيوية السابقة

قام بتحكيم البحث

أ.د / محمد منصور قاسم	**كلية الزراعة – جامعة المنصورة**
أ.د / شوقى محمود سليم	**كلية الزراعة – جامعة عين شمس**

Table(8). Phytochemical analysis, of *lemon*, orange and mandarin peel extracts

solvent / Ingredient	orange				lemon				Mandarin			
	Ethanol.	Eth.o.AC.	Acetone	Pet. Ether	Ethanol.	Eth.o.AC.	Acetone	Pet. Ether	Ethanol.	Eth.o.AC.	Acetone	Pet. Ether
Alkaloids	-	-	+	+	-	-	+	-	-	+	-	+
tannins	+	-	+	-	+	+	+	-	+	-	+	-
terpenoids	+	+	-	+	+	+	+	+	+	+	+	+
phlobatannins	-	-	-	+	+	-	+	-	+	+	+	+
Reducing sugars	+	+	+	-	+	+	-	-	+	-	+	+
flavonoids	-	+	+	+	-	+	+	+	-	+	-	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	-	+	-

+) indicates present (-) indicates absence(