

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

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ABSTRACT

The present study was carried out to find out the antimicrobial activity of ethyl acetate, acetone, ethanol and petroleum ether of lemon (*Citrus limone 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 O₁₅₇: H₇*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Candida albicans* and the results compared with the standard antibiotics, e.g. penicillin, chloramphenicol, 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, the least of the bacterial growth was obtained by using tube dilution method at 10 mg/ml concentration of different extracts against each *E.coli O₁₅₇:H₇* and *Staphylococcus aureus*. As shown, *Candida albicans* was 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 contains substances that can be used for therapeutic purpose or which are precursor of chemopharmaceuticals semi synthetic new drugs is 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 be potent antimicrobial agents like bacteria, fungus (Mathur *et al*; 2011). Antimicrobial activity of plants has been receiving attention many years ago as one of the most effective mechanisms 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 activities of essential oils, among which citrus oils, are also shown to be particularly interesting fields for applications within the food, cosmetic industries and antimicrobial (Caccioni, *et al.*, 1998). Herbs and spices with antimicrobial activity have been widely used both traditionally 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* O₅₇:H₇ ,*Listeria monocytogenes* and *Staph.aureus* by citrus Eos.scarcere 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* O₅₇:H₇ , *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 °C. Each extract were transferred to glass vials and kept at 4° 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 alkaloids: 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 O₅₇:H₇* , *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 inoculum:

Stock culture of *E.coli* O₅₇:H₇, *Staph. aureus*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* were cultured in nutrient broth at 37 °c for 24 hr for *E.coli* O₅₇:H₇ and *Staph,aureus* and at 30°c for 24hr for *Ps.flourescens* and *Ps. aeruginosa*. *Candida albicans* was cultured in Malt extract broth at 30°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* O₅₇:H₇ , *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* O₅₇:H₇ ,*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°c for 24hr for *E.coli* O₅₇:H₇ and *Staph. aureus* and 30°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* O₅₇:H₇ , *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* O₅₇:H₇ and *Staph,aureus* were incubated at 37°c for 24 hr on rotary shaker (100 rpm), the tubes for *Ps.flourescens* , *Ps. aeruginosa* and *C.albicans* were incubated at 30°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* O₁₂₇: H₇, *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* O₁₅₇: H₇ 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. albicans* 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
<i>E.coli</i> O ₁₅₇ :H ₇	10	12	11	15	10	12	10	12
<i>Staph aureus</i>	12	14	7	9	7	10	7	9
<i>Ps.flourescens</i>	7	10	7	10	7	9	8	10
<i>Ps.aeruginosa</i>	10	12	12	20	13	15	11	12
<i>C.albicans</i>	40	50	40	50	45	60	10	13

Data recorded in Table (2) showed that *E.coli* O₁₅₇: H₇ 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
<i>E.coli</i> O ₁₅₇ :H ₇	10	12	12	14	12	14	11	13
<i>Staph aureus</i>	10	12	9	12	11	13	9	11
<i>Ps.flourescens</i>	7	10	7	10	7	10	7	10
<i>Ps.aeruginosa</i>	12	15	14	17	18	20	18	25
<i>C.albicans</i>	50	70	30	35	35	60	40	45

Form the summerized results recorded in Table (3) it is evident that *E.coli* O₁₅₇: H₇ was more sensitive toward 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
<i>E.coli</i> O ₁₅₇ :H ₇	10	12	10	13	9	11	10	15
<i>Staph aureus</i>	10	12	9	11	8	12	8	10
<i>Ps.flourescens</i>	7	10	8	10	7	10	8	10
<i>Ps.aeruginosa</i>	15	35	17	30	12	25	18	20
<i>C.albicans</i>	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 nephelometric 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* O₁₅₇: H₇density 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		
<i>E.coli</i> O ₁₅₇ :H ₇	24	12	23	17	20	12	33	17	89	80
<i>Staph aureus</i>	15	9	20	7	16	10	22	10	83	77
<i>Ps.flourescens</i>	39	22	37	20	38	31	45	27	96	89
<i>Ps.aeruginosa</i>	27	19	32	25	37	26	40	25	93	85
<i>C.albicans</i>	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 O₁₅₇:H₇* 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		
<i>E.coli O₁₅₇:H₇</i>	17	10	0.8	0.5	0.6	0.3	13	0.8	89	80
<i>Staph aureus</i>	16	0.4	0.8	0.6	0.6	0.4	16	0.8	83	77
<i>Ps.flourescens</i>	26	17	25	0.9	14	9	31	20	96	89
<i>Ps.aeruginosa</i>	24	6	19	12	13	10	22	16	93	85
<i>C.albicans</i>	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 O₁₅₇:H₇*, *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 nephelometric turbidity (NTU).

Concentrations (mg/ml) microorganisms	Ethyle acetate		Acetone		Ethanol		Petroleum ether		Control A	Control B
	5	10	5	10	5	10	5	10		
<i>E.coli O₁₅₇:H₇</i>	11	5	0.9	0.6	0.5	0.2	12	0.6	89	80
<i>Staph aureus</i>	0.7	0.4	0.6	0.4	0.6	0.3	0.6	0.4	83	77
<i>Ps.flourescens</i>	30	18	13	7	15	8	20	16	96	89
<i>Ps.aeruginosa</i>	18	11	14	9	0.9	0.5	16	11	93	85
<i>C.albicans</i>	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 al.*, 2011). The antibacterial effects of the ethanolic 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 terpenes 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 Albicans* (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.aeruginosa* (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 O₁₅₇: H₇* and *Staph aureus* such exhibited diameter of inhibition zone 35 and 20 mm for *E.coli O₁₅₇: H₇* and *Staph aureus* respectively. Penicillin 10µg showed similar antimicrobial activity against each *Ps.flourescens* and *Ps.aeruginosa* in 25mm for each it *Ps.flourescens*and 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.albicans* 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
<i>E.coli O₁₅₇ :H₇</i>	20	18	35	10
<i>Staph aureus</i>	15	16	20	10
<i>Ps.flourescens</i>	25	-	-	20
<i>Ps.aeruginosa</i>	25	-	-	-
<i>C.albicans</i>	-	-	-	-

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 phytochemical.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

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CONCLUSION

Ethyl acetate, acetone, ethanol and petroleum ether extracts showed antimicrobial activity against *E.coli*O₁₅₇: H₇, *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.

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تقييم النشاط المضاد للبكتريا ووجود المركبات الفيتوكيميائية فى بعض المستخلصات
لقشور الموالح الليمون والبرتقال واليوسفى
أحمد فريد عبد السلام و فاطمة أحمد على مصطفى
المركز الإقليمي للأغذية والأعلاف – مركز البحوث الزراعية – مصر

اجريت الدراسة لمعرفة النشاط المضاد للبكتريا لمستخلصات ,ethyl acetate , acetone ethanol , petroleum ether لقشور الليمون والبرتقال واليوسفى على التركيزات (٥٠ او ٢٥ او ٥٠ مجم /مل)
تم عمل تحليلات النشاط المضاد للبكتريا باستخدام طريقة الانتشار والأنابيب المخففة ضد
E.coli O₁₅₇ H₇: Staphylococcus aureus ,pseudomonas flourescens ,pseudomonas aeruginosa, Candida albicans
قورنت النتائج بتاثير المضادات الحيوية القياسية مثل البنسللين والكلورامفينكول والأريثروميسين والكلوروتتراسيكلين
اظهرت النتائج ان المستخلصات على التركيزات المختلفة (٥٠ و ٢٥ و ٥٠ مجم/مل) من قشور الليمون والبرتقال واليوسفى عرضت المساحات الأمتل من التثبيط ضد *Candida albicans* باستخدام طريقة الانتشار وعلى الجانب الأخر فان اقل كثافة من النمو البكتيرى قد حصل عليها باستخدام طريقة الأنابيب المخففة على تركيز (١٠ مجم /مل) للتركيزات المختلفة ضد كل من *E.coli O₁₅₇ H₇: Staphylococcus aureus* وأوضحت النتائج ايضا ان *Candida albicans* كانت غير حساسة لكل المضادات الحيوية السابقة

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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(