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ANTIMICROBIAL ACTIVITY OF NATURAL ANTHOCYANINS AND CAROTENOIDS EXTRACTED FROM SOME PLANTS AND WASTES

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ABSTRACT

In the present study, the anthocyanins and carotenoids contents in some plants, herbs and either vegetables or fruit wastes were considered. Anthocyanins were extracted from red beet roots (*Beta vulgaris* L.), dark roselle petals (*Hibiscus sabdariffa* L.) and eggplant peels (*Solanum melongena*). Meanwhile carotenoids were extracted from spinach leaves (*Spinacia oleracea*), tumeric (*Curcuma Longa Zingibaraceae*) and tangerine peels (*C. reticulata Magnoliopsida*). These extracts were examined as antimicrobial agents against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella sp*., *E.coli, Trichoderma viride* and *Candida utilis.* The anthocyanins and carotenoids fractions in the extracts were quantitatively and qualitatively determined by HPLC and GC-MS and revealed the presence of great numbers of flavonoids and terpenoids compounds which have the ability to act as antimicrobial agents. Roselle extract proved the superiority over the other extracts against the tested microorganisms in exception with *Staphylococcus aureus*, whereas, the eggplant peels anthocyanins had the highest antibacterial action against it. However, red beet roots extract had negative effect against *Trichoderma viride*, *Candida utilis* and *Bacillus subtilis*. Similar effect of the inhibition zone values were observed between spinach and tangerine peels carotenoids toward the *Trichoderma viride* and *Salmonella sp.* Tangerine peels carotenoids were better than tumeric carotenoids toward *Candida utilis, Bacillus subtilis* and *Salmonella sp.*, while tumeric carotenoids were better than both tangerine peels and spinach carotenoids toward *Trichoderma viride, Staphylococcus aureus* and *E.coli.* Red beet roots anthocyanins showed inhibitory effect higher than that of spinach carotenoids against *Staphylococcus aureus, Salmonella sp* and *E.coli.* Similar effect was also recorded with tumeric carotenoids and roselle petals anthocyanins against *Trichoderma viride.* Also, nearlly inhibitory effect found by tumeric carotenoids and eggplant peels anthocyanins against *Bacillus subtilis.* Obtained data suggest the possibility to use such extracts in food processing to control pathogens and for food preservation as well.

Keywords : Antimicrobial, anthocyanins, carotenoids, red beet roots, spinach, roslle petals, tumeric, eggplant peels, tangerine peels and wastes.

INTRODUCTION

Anthocyanins and carotenoids are amongst the most utilized vegetable colorants in the food industry. Anthocyanins are one of the most important flavonoids that widely distributed group of water soluble natural pigments. They are responsible for the shiny orange , violet and blue colors in the flowers and fruits of some plants. However , anthocyanins are secondary products of plant metabolism and always exist in nature as glycosides. Glycosidic substitution increases both stability and water solubility. Flavonoid are a large group of naturally- occurring plant phenolic compounds that inhibit lipid oxidation by scavenging free radicals or by other mechanisms such as singlet oxygen quenching metal chelation and lipoxygenase inhibition. Moreover, in developing countries where medicines are quite expensive, investigation on antimicrobial activities from medicinal plants may still be needed (Gabriel and Jack, 2000 and Mohamed *et al*., 2009). Roselle (*Hibiscus sabdariffa* L.) has received considerable attention as a potential source of natural anthocyanins , pharmaceutical and cosmetics, currently, roselle is also claimed as a traditional medicine for kidney stones and can be used as an antibacterial and as antifungal substances. Antimicrobial berry compounds, especially dietary flavonoids may have important application in the future as natural antimicrobial agents for the food industry as well as for the field of medicine (Ruangsri *et al.*, 2008 and Burdulis *et al.*, 2009). Red beet accumulates betacyanins mainly betanin in the store root and the reactive oxygen species (ROS) are signals inducing the betacyanins synthesis that may act as ROS scavengers, limiting damage caused by wounding and bacterial infiltration as reported by Sepulveda *et al*. (2004).

Carotenoids are tetra terpenes, having 8 isoprene. The series of conjugated olefinic bonds constituents the chromophoric group of a carotenoid, which able to color food products from yellow to red. However, carotenoids are liposoluble and stable more than anthocyanins (Castaneda *et al.*, 2009). Citrus is one of the most important commercial fruit crops grown in all continents of the world and the peels of mature fruit is one of the richest and more complex sources of carotenoids. The antimicrobial activity of carotenoids extract displayed a variable degree of antimicrobial activity on pathogenic bacteria than that of lactic acid bacteria and they could used in dairy products and food processing to control pathogen,for preservation and alter the microbial ecology of the surface of a food (El-Badrawy and El-Fadaly, 2000, Nannapaneni *et al.*, 2008 and Tao *et al*., 2010).

Gur *et al.* (2006) assayed the tumeric extract as antimicrobial activity against various Gram-negative and Gram-positive bacteria. However, the antimicrobial activity was conducted by the agar well diffusion method and the plant extract showed various of antimicrobial activity on different tested microorganisms either eucaryotic or procaryotic cells.

So, the aim of this investigation is a trial to use some plants , herbs and either vegetables or fruits wastes as natural antimicrobial agents against six microorganisms chosen on the basis of their economic importance for human being.

MATERIALS AND METHODS

Materials:

Six raw materials, as natural sources of red, red violet, orange and yellow colorants, were used in this investigation. These tested materials namely were some plants such as red beet roots (*Beta vulgaris* L.*),* dark roselle petals (*Hibiscus sabdariffa* L.) and eggplant peels *(Solanum* *melongena)* as anthocyanins sources. Spinach leaves *(Spinacia oleracea)*, tumeric (*Curcuma Longa Zingibaraceae*) and tangerine peels **(***C. reticulata Magnoliopsida*) were used as carotenoids sources. These plants were purchased from Mansoura markets, Dakahlia, Egypt.

Microbiological experiments:

Microorganisms used

Six pathogenic microbial strains which used in this investigation were obtained from Microbiol. Dept., Damietta Faculty of Agric., Mansoura University. These strains were four bacterial strains namely *Staphylococcus aureus* (G+ coccoid shaped bacteria)*, Bacillus subtilis* (G+ long spore forming bacteria), *Salmonella sp* and *E.coli* (G- short rod bacteria). One fungal strain called *Trichoderma viride* and one yeast strain namely *Candida utilis.* These microbial strains were chosen for their economic importance regarding the human health.

Assessment of antimicrobial activities:

The plate diffusion methods was used here, holes with a cork borer were punched in medium seeded with a standard in column of 0.5 ml cell suspension in case of bacteria and yeast or 0.5 ml of spore suspension of fungi of young microbial culture using vortex mixer (No. 502550, Taiwan) under aspectic conditions. These cultivation media were nutrient agar (NA) or potato dextrose agar (PDA) in case of bacterial cells and yeast or fungi, respectively. A concentrated 0.05 ml of the tested natural pigment was put into the hole, left one hr to allow diffusion, then incubated at appropriate temperature and period of time. At the end of incubation period, the inhibition zones of microbial growth were measured and recorded (Bagamboula *et al.,* **2003).**

Methods:

Extraction of anthocyanins:

Anthocyanins were extracted from red beet roots ,karkadeh leaves and eggplant peels by using three different methods as described by Hamouda (1994), El-Bedawey (1973) and Todaro *et al*. (2009), respectively.

Colorimetric determination of extracted anthocyanins:

Anthocyanin pigments contents of the original extracts were determined colorimetrically according to the procedure described by Lees and Francis (2009) using the following equation:-

$$\text{Total (OD/100g)} = \frac{\text{Absorbance at 535 nm} \times \text{Diluted volume} \times \text{Total volume of the extract(ml)}}{\text{Volume of extract used for absorbancy measurement} \times \text{Weight of sample(gm)}} \times 100$$

Total anthocyanins (mg/100g) **=** Total OD/98.2

Fractionation and determination of anthocyanins :

The extracted anthocyanins were fractionated and determined by using HPLC in Central Laboratory of Food Technol. Res. Inst., Agric. Res. Center , Giza, Egypt.

Samples were analyzed by High Performance Liquid Chromatography (HPLC) Agilent 1100 series equipped with autosampler ,column compartment set at 45°C, quaternary pumb, variable wavelength at 520 nm and degasser set the flow rate of HPLC system to 1 ml/min across column C18 Hypersil BDS (4.6 × 250 mm, particle size 5 µm).

Extraction of carotenoids :

Carotenoids were extracted from tumeric, tangerine peels and spinach by using three different methods as described by Braga *et al.(*2003), Zein *et al.* (2004) and Bunea *et al*. (2008), respectively.

Colorimetric determination of extracted carotenoids:-

Total carotenoids were determined colorimetrically according to the method of Tao *et al*. (2010) using the following equation :-

$$\text{Total carotenoids mg/g (d.w.)} = \frac{V(A-0.0051)}{0.175W}$$

Where:-

A : Is the absorbance value of diluted extraction at 450nm.

V: Is the final volume of extract.

W(g): Is the weight of dried powder.

Fractionation and determination of carotenoids :

The extracted carotenoids were fractionated and determined by using Gass Chromatography – Mass Spectroscopic (GC-MS) in Central Agric. Pesticides Laboratory, Agric. Res. Center, Dokki-Giza, Egypt.

GC-MS analysis was performed with an Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5MS (30 m × 320 µm × 0.25 µm film thickness). Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1.0 ml/min., pulsed split less mode. The solvent delay was 3 min. and the injection size was 1.0 µl. The GC temperature program was started at 60° C (3 min) then elevated to 260° C at rate of 8° C/min. the detector and injector temperature were set at 280 and 250° C, respectively. Wiley7Nist05 and Nist05 mass spectral data base was used in the identification of the separated peaks.

Spectrophotometric analysis:

The highest absoptions occurred in both extracted anthocyanins and carotenoids were examined at the Spectral Analysis Unit by using UNICAM UV/VIS Spectrometer UV.2.Chemistry Department, Faculty of Science, Mansoura University., Egypt.

RESULTS AND DISCUSSION

Extraction and quantitative determination of anthocyanins and carotenoids:-

A preliminary study was carried out to determine the anthocyanins pigments extracted from red beet roots (*Beta vulgaris* L.*),* dark roselle petals (*Hibiscus sabdariffa* L.) and eggplant peels *(Solanum melongena)* and carotenoids extracted from tumeric (*Curcuma Longa Zingibaraceae*), tangerine peels (*C. reticulata Magnoliopsida*) and spinach leaves *(Spinacia oleracea)* to evaluate these raw materials as sources of natural antimicrobial agents.

Data presented in Table (1) show the contents of anthocyanins extracted from red beet roots, roselle petals and eggplant peels (190.2, 617.8 and 115.3 mg/100g, respectively).These results are in accordance with the results given by Harmer (1980), who found that the extraction of red pigment from beet roots produced low yield and a large proportion of sugar.

From the same Table, it can be clearly observed that tumeric had more content of carotenoids followed by tangerine peels and spinach leaves to be 18.6 & 17.3 and 9.7g/100g, respectively. These results are almost near to those of Gross(1977) and Jayaprakasha *et al.*(2002).

Table(1) : Anthocyanins and carotenoids pigments contents of some raw materials (D.W.):

Pigment / Sources	Anthocyanins (mg/100g)	Carotenoids (g/100g)
Red beet roots	190.2	–
Roselle petals	617.8	–
Eggplant peels	115.3	–
Spinach	–	9.7
Tumeric	–	18.6
Tangerine peels	–	17.3

Identification of the individual fractions:-

The maximum absorptions recorded with red beet roots anthocyanins at 470 - 536 nm, beside other absorptions at 380 and 450 nm. These results indicated that red beet roots were rich in other components such as flavonoids. These results are in agreement with those obtained by Harborne (1973), who found that flavonoids contained conjugated aromatic systems and thus observed intense absorption bands in UV and visible regions of the spectrum like anthocyanins which reflect the red to blue range of the visible spectrum 475–560 nm. The maximum absorptions recorded with roselle petals and eggplant peels anthocyanins were at wave length ranged between 535 - 546 and 544 nm, respectively. On the other hand, the maximum absorptions for carotenoids extracted from spinach were 296, 414, 466 and 660 nm. Meanwhile, the highest absorptions recorded with tumeric carotenoids were at wave lengths 400, 426 and 440 nm. These results are in accordance with those of Xu and Wang (2004), who found that curcumin gave the maximum absorption at 426 nm and it was the predominant component in the carotenoids extracted from tumeric powder .

The characteristic visible light absorption spectrum of carotenoid pigments is due to the system of conjugated double bonds of their hydrocarbon chain (polyene).The maximum absorption of carotenoids usually appeared between 400 to 500 nm (Weedon and Moss,1995). The maximum absorptions for extracted carotenoids from tangerine peels were found at 429, 439, 456 and 467 nm.

Fractionation and determination of anthocyanins using High Performance Liquid Chromatography (HPLC):

The extracted anthocyanins from red beet roots, roselle petals and eggplant peels were fractionated and determined using HPLC and data obtained are shown in Figures (1, 2 and 3).

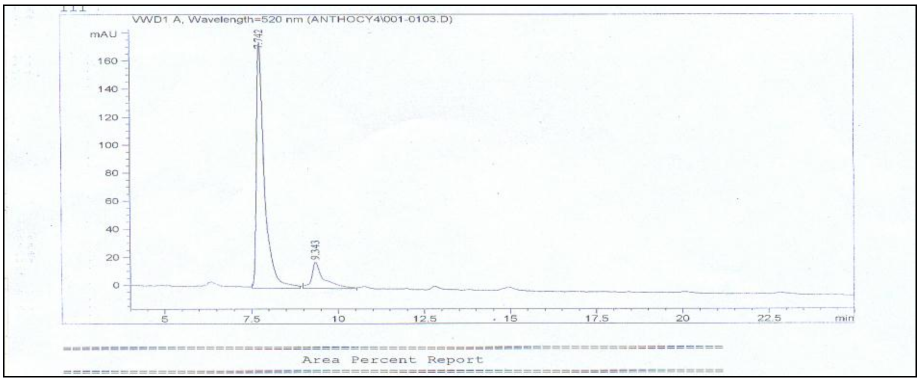

Fig. (1): HPLC fractions identified in the extracted red beet roots anthocyanins

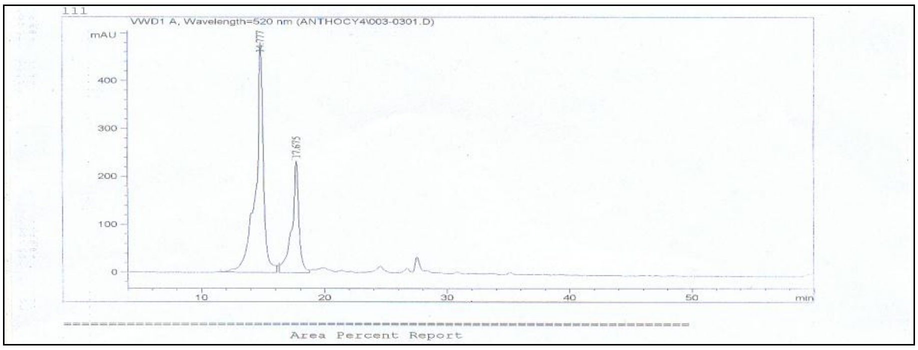

Fig.(2): HPLC fractions identified in the extracted roselle petals anthocyanins

Fig.(3): HPLC fractions identified in the extracted eggplant peels anthocyanins

The acid hydrolysis HPLC method greatly simplifies the anthocyanins profile in the extracted anthocyanins and converts anthocyanins into anthocyanidins aglycones. Retention times and spectra were compared to those of pure standards.

Regarding the fractions of red beet roots anthocyanins, there were two main fractions detected namely pelargonidin (85.5 %) at Rt being 7.742 and malvidin (14.5%) at Rt to be 9.343 (Fig.1). Meanwhile, the fractions detected in roselle petals sample namely, delphinidin (69.4%) at Rt equal to 14.777 and cyanidin (30.6%) at Rt to be 17.675 (Fig.2). These data are in agreement with those obtained by Badawy (2007) and Ruangsri *et al*. (2008), who reported that delphinidin was the major pigment responsible for the reddish violet color. Cyanidin was also presented in little amounts of delphinidin. The relative proportion of delphinidin and cyanidin was 70.9% and 29.1%, respectively.

On the other hand, the fractions detected with the eggplant peels sample were delphinidin (90.7%) at Rt to be 14.855 and petunidin (9.3%) at Rt (8.592). So, the predominant anthocyanins fraction detected in red beet roots sample was the pelargonidin, while, delphinidin was the main anthocyanins in both roselle petals and eggplant peel samples in concentrations of 69.4 and 90.7%, respectively (Fig.3).

Fractionation and detremination of carotenoids by Gass Chromatography-Mass Spectroscopic (GC-MS):

The extracted carotenoids from tumeric , tangerine peels and spinach was fractionated and determined by using GC-MS and results are shown in Figures(4, 5 and 6) .

In the present study, the carotenoids were extracted from spinach are shown in (Fig.4). The major fraction identified carotenoid was lutein (26.6%) followed by beta carotene (13.9 %) at Rt being 21.5 and 19.42 min , respectively. These data are in agreement with Bunea *et al*. (2008) who found the values of lutein in spinach were in the range 41–59 mg/kg (wet basis) suggesting its predominant presence compound to the other carotenoids. Moreover, after the different treatment applied to the spinach samples, lutein was the most stable carotenoid to be 37 – 46 % of total carotenoid content. Azevedo-Meleiro and Rodriguez-Amaya (2005) indicated that the carotenoids concentrations in mature spinach leaves from beta-carotene, lutein, violaxanthin and neoxanthin were from two to four times greater than those of the young leaves.

Both of zeaxanthin and neoxanthin was represented in the examined carotenoids extracted from spinach in concentration of 8.1 % at Rt to be 19.13 and 22.23 min, respectively. Lycopene was also recorded (3.1 %) at Rt 19.93 min.

Also, the methyl esters of both palmatic acid (as fatty acid C:16) and cinnamic acid (as phenolic acid) were found in the spinach carotenoids in percentage ranged from 3.34 to 7.41 %. The fatty acid C: 14 (myrestic acid) was detected in concentration of 0.97 % at Rt being 18.14 min. On the other hand, the GC-MS announced about the presence of high concentrations from other phenolic acids derivatives which reached to about 8.273 %.

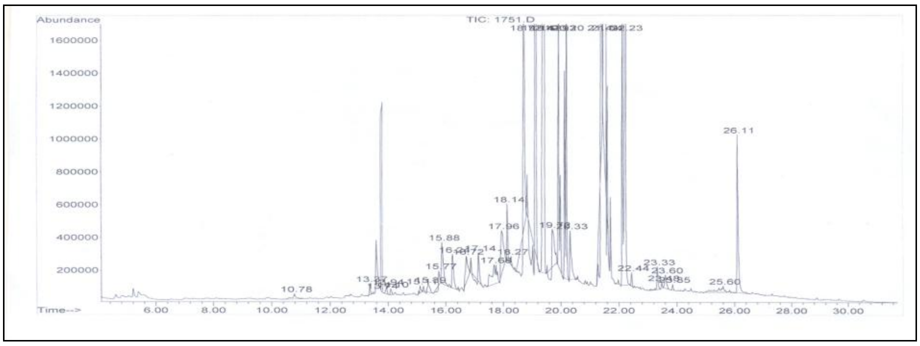

Fig.(4) : GC-MS of carotenoids extracted from spinach leaves.

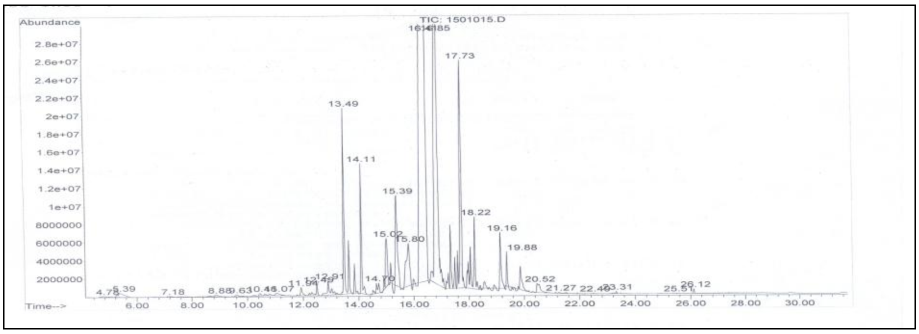

Fig.(5): GC-MS of carotenoids extracted from tumeric.

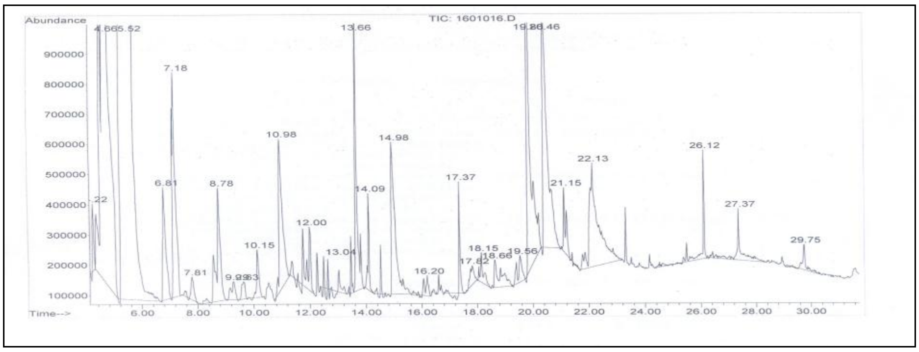

Fig.(6) : GC-MS of carotenoids extracted from tangerine peels.

From Fig. (5), it could be noticed that there were more than 28 compounds were characterized in carotenoids extracted from tumeric and they could be classified into four groups; carotenoids fractions, fatty acids derivatives, phenolic derivatives and terpenes. The main carotenoids fractions detected in the tumeric extract were beta–tumerone ,apha-tumerone and alpha curcumin at Rt to be 16.41, 16.85 and 13.49 min in concentrations of 44.9, 19.8 and 5.1%, respectively. These data are in agreement with those of Stankovic (2004), who reported that the predominant coloring principles in tumeric were sesquiterpene ketones:

alpha-tumerone, beta-tumerone and aromatic-tumerone. On the other side, 4-hydroxycinnamoyl (feruloyl) methane exhibited area peak around 12.9 min in concentration of 0.8%. That compound is consider one of the principal colouring components of curcumin. Curcumin is a colouring substance obtained by solvent extraction of tumeric and it is an orange-yellow crystalline powder (Ahn and Kay, 2007). Also, limonine, heptanol, caryophyllene, beta-sesquiphellandrene and pyridine were terpenoids and diarylheptanoid detected under the GC-MS instrumental conditions with concentrations of 0.5, 0.1, 0.4, 2.4 and 3.5, respectively. These terpenoids detected at area peaks around 5.39 ~ 15.38 min. These date are in agreement with Akiyama *et al.* (2006) who isolated new terpenoids and diarylheptanoid from rhizomes and *Zingiber ottensii.*

Compounds detected in the carotenoids extracted from tangerine peels could be divided into two groups ; hydrocarbons and oxygenated compounds. The main hydrocarbons were limonene (90.8%) followed by beta-mercene (2.831 %) at Rt to be 5.51 and 4.66 min, respectively (Fig.6). These are in agreement with Di Pasqua *et al*. (2006) and Nannapaneni *et al.* (2008), who reported that the principal hydrocarbon in citrus is limonene, which composed of non polar compounds that comprise more than 95 % of most citrus and can be distilled to produce a nearly odorless compound. Meanwhile, the oxygenated compounds are polar and less than 5% of the volume and contain esters, aldehydes, ketones, alcohols, phenols and oxides.

Values of microbial inhibition activity of anthocyanins and carotenoids extracts:

The antimicrobial activity of some natural anthocyanins and carotenoids extracted from some plants, herbs and wastes against the microorganisms employed and its activities were qualitatively assessed by the presence or absence of inhibition zones and obtained zone diameters were measured after incubation at 37° C.

Red beet roots, roselle petals and eggplant peels were used as natural sources of anthocyanins extracts. Meanwhile, spinach leaves, tumeric and tangerine peels as sources of carotenoids extracts. Applying theses plants, herbs and wastes pigment extracts against four strains. These strains were four bacterial strains namely *Staphylococcus aureus, Bacillus subtilis, Salmonella sp* and *E.coli.* One fungal strain called *Trichoderma viride* and one yeast strain namely *Candida utilis*. The obtained inhibitory effects are shown in Table (2) and Fig. (7) .

Illustrated data proved the superiority of roselle petals anthocyanins over the other pigment extracts against the tested microbes in exception with *Staphylococcus aureus,* whereas, the eggplant peels anthocyanins was the highest antibacterial activity against *Staphylococcus aureus* with inhibition zone of 3.55 Cm. That was may be due to delphinidin which detected by the HPLC in both roselle petals and eggplant peels anthocyanins in percentages of 69.4 and 90.7%, respectively. The presence of cyanidin (30.6 %) in roselle petals anthocyanins extract led to synergist the role of delphinidin as antibacterial and antifungal against all other tested strains. These data are in agreement with those obtaines by Ruangsri *et al.* (2008) who confirmed that roselle petals can be used as antibacterial and as antifungal substance to their content from delphinidin and cyanidin. Also, delphinidin and cyanidin which detected in the ethanolic extract of *P. ientiscus* and *P. Atlantic* by TLC were played important role as antimicrobial agents (Benhammou *et al .,* 2008).

From the Table (2) and Fig. (7), it could be noticed that red beet roots anthocyanins extract has negative effect as antifungal and as antibacterial against *Trichoderma viride & Candida utili*s and *Bacillus subtilis*, respectively. On the other side, red beet roots anthocyanins showed inhibitory effect more better than spinach carotenoids against all the other examined procaryotic cells including *Staphylococcus aureus, Salmonella sp* and *E.coli.* That may be due to the pelargonidin which detected in red beet roots anthocyanins by HPLC in a high concentration reached to 85.5% and also to malvidin (14.5%). These data are in agreement with Naz *et al*. (2007), who found that the antimicrobial activity of pomegranate fruit led to the pelargonidin which exhibited substantial activity against species of *Staphylococcus aureus, Salmonella sp* and *E.coli.* However, pelargonidin was more active against Gram-positive species.

Buradulis *et al.* (2009) noticed that the results obtained by chromatography analysis to bilberry and blueberry anthocyanins indicated that cyanidin is a dominant anthocyanidin in bilberry and malvidin in blueberry samples revealed antimicrobial properties. Listed data in Table (2) showed similar effect in the zone values were observed between spinach and tangerine peels carotenoids when they examined as antifungals toward the *Trichoderma viride* as well as against *Salmonella sp*. That effect may be returned to the presence of several important compounds which detected by GC-MS in both tangerine peels and spinach carotenoids that play an observable antimicrobial action. Whereas, limonine (90.8 %) found in tangerine peels carotenoids and lutein (26.6%), beta-carotene (13.9%), zeaxanthin (8.1%), neoxanthin (8.1%), lycopene (3.1%), fatty acids, phenolic acids (e.g.cinnamic acid) and their derivatives in spinach carotenoids.

Azevedo-Meleiro and Rodriguez-Amaya (2005) and Wang *et al.* (2007) indicated that spinach and other dark green leafy vegetables were the lutein rich sources of beta-carotene and the carotenoids concentrations in mature spinach leaves from beta-carotene, lutein, violaxanthin and neoxanthin were from two to four times greater than those of the young leaves. Organic acids, phenolics and flavonoids possess antimicrobial activity since they were responsible for enhance the resistance of fruit tissues to deterioration and spoilage. However, cinnamic acid delay the spoilage of kiwi fruit at chilling temperatures without adversely affecting sensory quality. In citrus peel , there is a natural compound namely, limonine and it has potent antimicrobial and inhibit the growth or kill the pathogenic bacteria and could play a natural alternative for chemical in food preservation (Nannapaneni *et al*., 2008 and Tao *et al.*, 2010) and that may explain the higher inhibitory effect to the tangerine peels carotenoids than spinach carotenoids against *Staphylococcus aureus, Bacillus subtilis* and *E.coli* as well. Tangerine peels carotenoids were also better than tumeric carotenoids toward *Candida utilis*, *Bacillus subtilis* and *Salmonella sp*. Tumeric extract had little effect on

Bacillus subtilis (Gur *et al.*, 2006). Meanwhile, it could be noticed that tumeric carotenoids were better than both tangerine peels and spinach carotenoids toward *Trichoderma viride*, *Staphylococcus aureus* and *E.coli*. these data are in disagreement with those of Gur *et al.* (2006) who showed that tumeric extract was not effective against *E.coli* and it was most effective just on *Staphylococcus aureus*.

Meanwhile, obtained data are in agreement with those obtained by Akiyama *et al*. (2006) and Norajit *et al*. (2007) who showed that the major component of tumeric is tumerone and that compound had strong antibacterial activity against *Staphylococcus aureus E.coli*, *Bacillus cereus* and *Listeria monocytogenes.* In the current study , alpha and beta tumerone were detected by GC-MS in tumeric carotenoids in percentages of 19.8 and 44.9 %, respectively. Some minor linoleic acid and fatty acids derivatives which could enhance the antifungal and antibacterial activity of tumeric carotenoids. Percentages of the main types of fatty acids which were identified by GC-MS in ethanol extracts from tumeric enhanced the antimicrobial effect of tumeric extract against Gram-positive bacteria such as *Staphylococcus aureus* which cause inflammation and food poisoning (Gur *et al*., 2006). On the other side, there were similar effects recorded in the zone values between tumeric carotenoids and roselle petals anthocyanins when their activity as antifungal agents examined against *Trichoderma viride* and nearlly the same zone values between tumeric carotenoids and eggplant peels anthocyanins against the *Bacillus subtilis* as G+ spore forming long bacilli.

Mohamed *et al*. (2009) explained the growth inhibition caused by the anthocyanins and carotenoids extracts to the tested fungi, yeast and pathogenic bacterias. Flavonoids are known to be synthesized by plants in response to microbial infection and their antimicrobial activity is probably due to their ability to complex with bacterial cell walls. Also, lipophilic flavonoids may disrupt the microbial membranes.

Finally, it could be concluded the possibility to use such extracts in food processing to control pathogens and for food preservation as well.

Table(2): Values of inhibition zones of examined microorganisms as affected by extracted pigments Values expressed the diameter of inhibition zone in Cm

Extracted pigment	Examined microorganisms					
	Eucaryotic cells		Procaryotic cells			
			*G+		**G-	
	Trichoderma viride	***Candida utilis***	***Staphylococcus aureus***	***Bacillus subtilis***	***Salmonella sp***	***E.coli***
Red beet roots	Negative	Negative	1.68	Negative	2.20	1.63
Roselle petals	3.00	4.23	1.62	3.93	4.98	4.83
Eggplant peels	1.67	1.83	3.55	2.13	3.05	3.03
Spinach	1.67	1.93	1.50	1.67	1.58	1.40
Tumeric	3.00	2.17	2.80	2.17	1.50	2.07
Tangerine peels	1.67	3.50	1.55	2.9	1.58	1.92

*** G+:Gram positive cells**

**** G-: Gram negative cells**

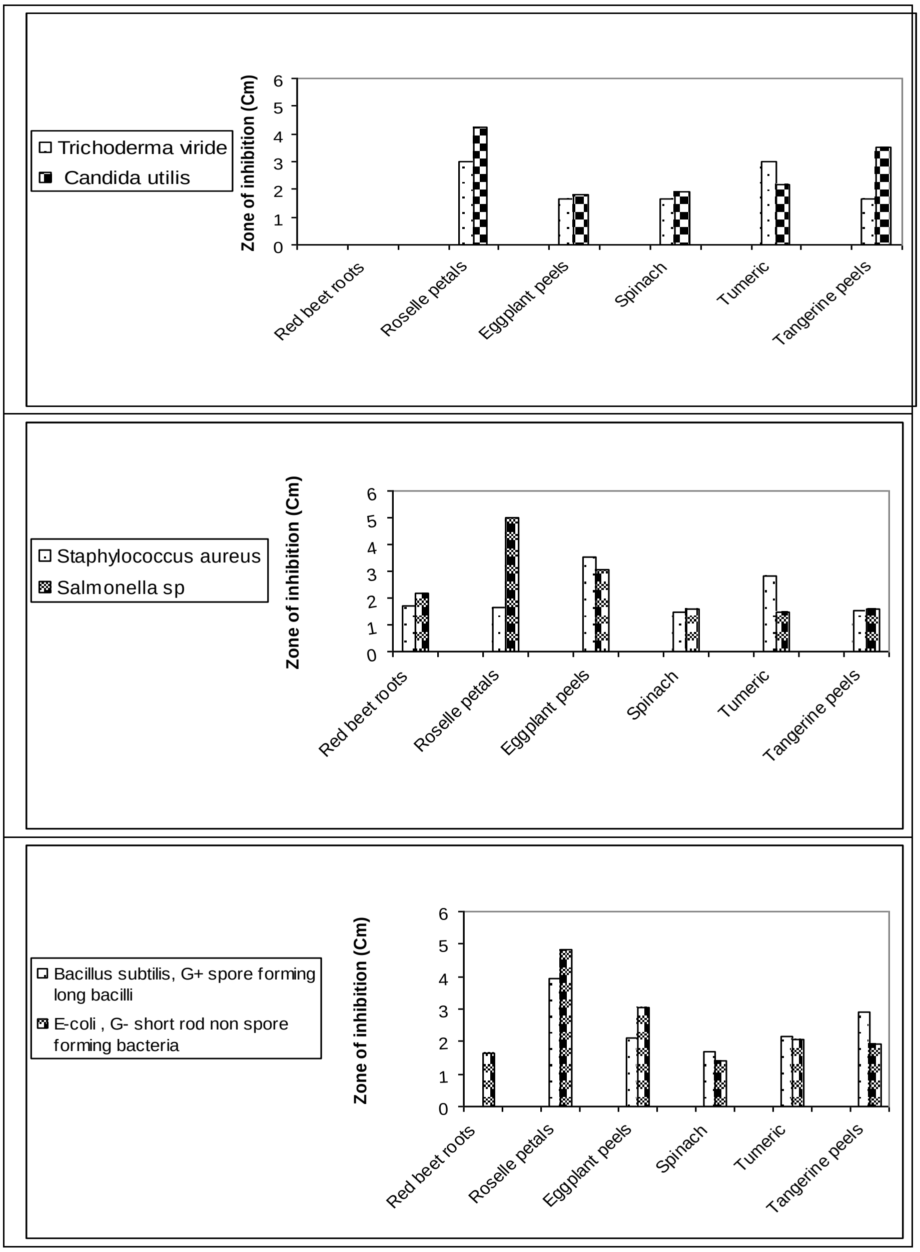

Fig. (7): Values of growth inhibition of tested microbsby natural anthocyanins and carotenoids extracts.

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النشــاط المضــاد للميكروبــات لصــبغات الأنثوســيانينات والكاروتينويـدات الطبيعيــة المستخلصة من بعض النباتات والمخلفات

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في هذه الدراسة تم تقدير محتوى بعض مستخلصات النباتات ، الأعشاب ومخلفات الخضر والفاكهة من الأنثوسيانينات والكاروتينويدات. تم الحصول على الأنثوسيانينات من جذور البنجر ، بتلات الكركديه ، وقشور الباذنجان ، والكاروتينويدات من أوراق السبانخ ، ريزومات الكركم ، وقشور اليوسفي ثم تم دراسة الدور الذي يمكن أن تقدمه مثل هذه الصبغات كمضادات لنمو الميكروبات ذات الأهمية الاقتصادية مثل بكتريا *Staphylococcus aureus* ,*Bacillus subtilis*, *Salmonella sp* , *E.coli* وفطر *Trichoderma viride* وخميرة *Candida utilis*

أظهرت نتائج التحليل الكروماتوجرافى لهذه المستخلصات عن وجود عدد هائل من الفلافونويدات والتربينات ذات الفعل المضاد للنمو الميكروبي ، ووجد أن أفضل هذه المستخلصات هو مستخلص أنثوسيانينات بتلات الكركديه لما حققه من تثبيط ملحوظ تجاه جميع الميكروبات الممرضة المختبرة، ماعدا مستخلص أنثوسيانينات قشور الباذنجان الذي حقق تثبيطا أعلى تجاه ميكروب ال *Staphylococcus aureus* ، بينما أعطى مستخلص أنثوسيانينات جذور البنجر نتائج مرضية مع جميع الميكروبات المختبرة وسالب التأثير على كل من :

بكتريا *Bacillus subtilis* وفطر *Trichoderma viride* وخميرة *Candida utilis*. من ناحية أخرى فقد تعادل تأثير كاروتينويدات أوراق السبانخ وقشور اليوسفي في تثبيط النمو الميكروبي لكل من بكتريا *Salmonella sp* وفطر *Trichoderma viride* بينما حققت كاروتينويدات قشور اليوسفي معدلات تثبيط أعلى مما حققه مستخلص كاروتينويدات الكركم تجاه نمو بكتريا *Bacillus subtilis,* *Salmonella sp* ، وخميرة *Candida utilis* في حين استطاع مستخلص الكركم التفوق بشكل أكبر من مستخلص أوراق السبانخ وقشور اليوسفي على نمو بكتريا *Staphylococcus aureus* and *E.coli* وفطر *Trichoderma viride* وبمقارنة مستخلصات الأنثوسيانينات بمثيلاتها من الكاروتينويدات كمثبطات لنمو الميكروبات تحت الدراسة وجد أن لمستخلص جذور البنجر القدرة بشكل أكبر من مستخلص السبانخ في السيطرة على نمو بكتريا *Staphylococcus aureus*، *Salmonella sp* و*E.coli* بينما تعادل تأثير كل من مستخلصي بتلات الكركديه وريزومات الكركم تجاه تثبيط نمو فطر *Trichoderma viride* في حين أعطى مستخلص قشور الباذنجان نتائج قريبة من نتائج مستخلص الكركم كمثبطات لنمو بكتريا *Bacillus subtilis* . لذلك تقترح النتائج المتحصل عليها في هذا البحث إمكانية استخدام مثل هذه المستخلصات في مجال التصنيع الغذائي للحفظ وللحد من نمو البكتريا الممرضة للإنسان.

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