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Antibacterial and antioxidant activities of *Physalis peruviana* and *Hyphaene thebaica* extracts

Sarah Mokhtar Abd-ELmageed*1, Hala Mohamed Abushady2 and Afaf Ali Amin1

1. National Nutrition Institute, Food Hygiene Department, Microbiology Unit, Egypt 2. Ain Shams University, Microbiology Department, Science faculty, Egypt

*sarahmokhtar.505@gmail.com

ABSTRACT

Ethanol extract of *Physalis peruviana* and water extract of *Hyphaene thebaica* were tested for their inhibitory effects on eight bacterial strains (*Escherichia coli, Proteus mirabilis, Salmonella typhimurium, Salmonella entrica*, *Shigella dysenteriae*, *Bacillus cereus, Bacillus licheniformis* and *Staphylococcus aureus*) by using well diffusion technique. The study demonstrated that *Staph. aureus* was the most sensitive gram-positive strain and *E. coli* was the most sensitive gram-negative strain to ethanol extract of *Physalis peruvianae* and water extract of *Hyphaene thebaica*. The inhibition zone diameters near to the synthetic antibiotic (ciprofloxacin). The antioxidant activity of the both extracts was also investigated by using 2, 2 - diphenyl, 1- picrylhydrazyl (DPPH) scavenging activity and ascorbic acid as control. The results showed that the scavenging effects of both extracts on DPPH radicals increased by increasing the concentration. The ethanolic extract of *Physalis peruvianae* and water extract of *Hyphaene thebaica* were a promising source of natural antimicrobial agent, antioxidant agent and as natural preservative of processed food.

Key word: *Physalis peruvianae*, *Hyphaene thebaica*, Antimicrobial activity, DPPH radical scavenging.

INTRODUCTION

Treatment with synthetic antibiotics is not always possible due to their high cost, development of widespread antibiotic resistance among the pathogens and undesirable side effects associated with the continued use of synthetic drugs (Kaur et al., 2010). Many people prefer to use preparations obtained from plants growing in their countries following folk tradition for medication alternative (Fabiola et al., 2002). Plant products may be used as antibiotic alternatives and do not cause resistance in bacteria (Arben et al., 2018).

The medicinal values of these plants lie in their polyphenolic components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid and phenolic acids (Shariff, 2001).

Physalis peruviana belongs to the family Solanaceae and genus Physalis (Cedeño and Montenegro, 2004). It is a climacteric fruit grown in countries such as Peru, Venezuela, Egypt, South Africa, and Australia (Narváez Cuenca et al., 2014). It has been shown to provide significant health benefits because of its therapeutic properties such as anti-pyretic, antiinflammatory, anti-allergic, anti-ulcer, antimicrobial anti-oxidant and (Tammu and Ramana, 2015). It was reported that the ethanolic extract of its fruit had antibacterial and antioxidant properties and it is used for treating diseases like cancer, leukemia, malaria, hepatitis, dermatitis asthma, and rheumatism Sathyadevi and Subramanian (2015)

Hyphaene thebaica is a desert palm native to Egypt, Sub-Saharan Africa and West India. It is called African doum (Dosumu *et al.*, 2006). It belongs to the

family Arecaceae (Idrees and Mohammed, 2015). Various extracts of *H. thebaica* are being used in the treatment of bilharzia, haematuria, after child birth bleeding, and also as a haematinic agent (Adaya *et al.*, 1977). The antimicrobial and antioxidant activities are shown greatly in the aqueous extract of the Doum fruit because of its luxurious amounts of water soluble phenolic contents (Hassan *et al.*, 2018).

MATERIALS AND METHODS I- Materials:

1-Media and chemical reagents

- Nutrient agar (Oxoid) (Lapage *et al.*, 1970).
- Nutrient broth (Oxoid) (Bolton *et al.*, 1984).
- Peptone water (Himedia): It used for bacteriological analysis.
- Ethyl alcohol 70% (El-Nasr Pharmaceutical Chemical Company)
- Ciprofloxacin discs: as antibiotic
- 0.5 McFarland standard (Biomerieux)
- Ascorbic acid, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

2-Plant materials

Fresh ripened fruits of *Physalis* peruvianae (golden berry) and *Hyphaene* thebaica (Doum) were purchased in March 2015 from local markets in Egypt. The investigations were performed at Food Safety Department, Microbiology Unit in National Nutrition Institute, Cairo, Egypt.

3- Identified bacterial strains

aureusATCC Staphylo coccus 29213, Salmonella typhimorium ATCC 14028, Proteus mirabilis ATCC 43071, E. coli **ATCC** 10536, **Bacillus** licheniformis ATCC 14580, Bacillus cereus ATCC 10876 were obtained TCS bioscience LTD, Botolph from Clydon, Buckingham, MK 18 2LR, England. Salmonella entrica (Subspp. entricasero var Tennyson) and Shigella dysenteriae (Sub group A) proved by Hygiene Institute/National *Salmonella* Center, (Federal German Republic). All strains were stored in nutrient agar stabs, sealed and kept in refrigerator and subcultured every three months.

II-Methods

1-Preparation of different extracts:

According to Jaca and Kambizi (2011) with slight modifications, for Physalis peruviana, the husks were carefully removed from the fruit by hand. Then the fruit samples were washed with tap water then by distilled water and gently wiped with paper tissue. Then they were air dried without direct exposure to the sun to avoid evaporation of some active compounds and ground into powder using a laboratory blender. Ten grams of golden berry powder were added in a flask with 100 ml of ethanol 70% to obtain ethanolic extract of *P. peruviana*. Then this extract incubated at room temperature for 24 hours. The extract was filtrated by (Whatman no.1) and then the filtrate was concentrated at 40°C using oven till getting ride off solvent and having solid residue, which stored in freezer at -20°C till use and rehydrated by water for further uses.

According to Aamer (2016) with slight modifications, fruits of Doum were washed by tap water then by distilled water and then were drained. The external part and the edible portion were crushed after scraping from the seed using stainless steel knife. The crushed portions were air dried, and then they were oven dried at about 45°C for three days. The dried portions were milled using electrical blender to obtain fine powder. Ten grams of Doum powder were added in a flask with 100 ml of water to obtain water extract of *Hyphaene thebaica*. Then incubated at room temperature for 24 hours. The extract was filtrated by (Whatman no.1) and then the filtrates were concentrated at 40°C using oven till getting ride off solvent and having solid residue, which stored in freezer at -20°C till use and rehydrated by water for further uses.

2-Preparation and standardization of inoculum:

The tested bacteria were inoculated on nutrient broth and incubated at 37°C for 24 hours. The turbidity was adjusted to 0.5 McFarland turbidity standard (Doughari and Manzara, 2008).

3-Preparation of extract concentrations:

One gram of each extract put in 10 ml sterile distilled water and shaked well until dissolved under aseptic condition to prepare 10% concentration. The previous steps repeated by 3 and 5 g. of each extract in 10 ml sterile distilled water to make concentrations (30%) and (50%), respectively.

4-Screening of antibacterial activity a-Well diffusion technique

To observe the inhibitory spectrum of golden berry (*Physalis peruviana*) and Doum fruit (*Hyphaen ethebaica*) extracts against eight pathogenic bacteria. The screening of antibacterial activity was assessed based on the diameter of the clear zone surrounding the well (including the well diameter) in millimeter (mm). The tests were conducted in triplicates (Aboaba *et al.*, 2006) and (Nurmahani *et al.*, 2012).

b- Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) for each extract:

The lowest concentration that did not permit any visible growth was considered as MIC. MBC was considered as the lowest concentration that could not produce any growth (Aboaba *et al.*, 2006).

5-Determination of antioxidant activity:

The antioxidant activity of plant material was assayed by DPPH assay. Freshly prepared (0.004% w/v) methanol

solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10C° in the dark. A methanol solution of the tested extracts was prepared. A 40μL aliquot of the methanol solution was added to 3ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Yen and Duh, 1994). The absorbance of the DPPH radical without antioxidant as control and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates.

The percentage inhibition (PI) of the DPPH radical was calculated according to the formula: (Yen and Duh, 1994) $PI = [\{ (AC - AT)/AC \} \times 100]$ Where , AC = Absorbance of the control at t = 0 min

AT = Absorbance of the sample + DPPH at t = 16 min

6-The statistical analysis

Arithmetric mean±SD, average and standard deviation were calculated using SPSS Statistical Package version 21 and the results were tabulated by Harvard graphics packages version 4 (1998), for representing the results graphically. A significant *P*-value was considered when *P* is less than 0.05 (Jayawardana *et al.*, 2015).

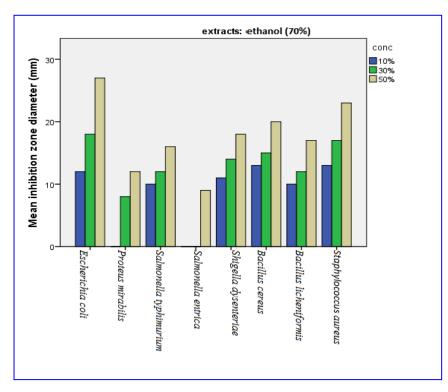
RESULTS

Table (1) and Figure (1) showed that the most sensitive microorganism for ethanol (70%) extract of *Physalis peruvianae* was *Escherichia coli* with the highest mean value (19.0) followed by *Staphylococcus aureus* with mean value (17.67). Also, there was high significant difference between 10, 30 and 50 % concentrations with *P-value* (0.008).

Table (1): Antibacterial activities of ethanol (70%) extract of *Physalis peruvianae* expressed as inhibition zone diameter (mm) at different concentrations (10, 30 and 50%)

Bacterial species	Conc. of Ethanol (70%) extract			Mean ±SD
	10%	30%	50%	
Escherichia coli	12	18	27	19.0±7.6
Proteus mirabilis	-	8	12	6.67±6.1
Salmonella typhimurium	10	12	16	12.67±3.1
Salmonella entrica	-	-	9	3.00±5.2
Shigella dysenteriae	11	14	18	14.3±3.5
Bacillus cereus	13	15	20	16.0±3.6
Bacillus licheniformis	10	12	17	13.0±3.6
Staphylococcus aureus	13	17	23	17.67±5.03
Mean ±SD	8.63±5.44	12.0±5.8	17.75±5.	12.79±6.64
			7	
P- value	0.008**			

^{*}P < 0.05 (Significant), **P < 0.01 (Highly significant), (-) no inhibition



Bacterial species

Fig. (1). Mean inhibition zone diameter of each bacterial species at three concentrations (10, 30, 50%) for ethanolic extract of *Physalis peruvianae*

Table (2) and Figure (2) for the water extract of *Hyphaene thebaica* showed that the most sensitive microorganism for was *Staphylococcus*

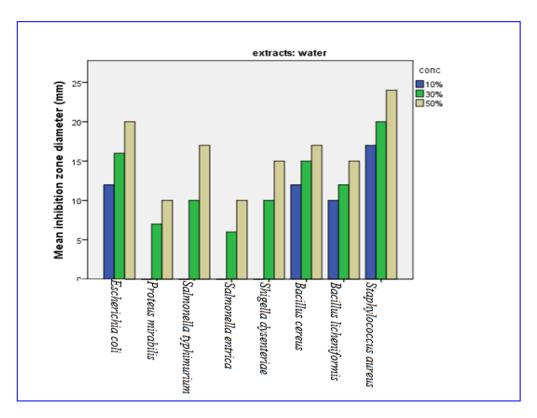
aureus with the highest mean value (20.33) followed by *Escherichia coli* with mean value (16.00) also there was high

significant difference between 10, 30 and 50 %concentrations with *P-value* (0.009).

Table (2): Antibacterial activities of Water extract of *Hyphaene thebaica* expressed as inhibition zone diameter (mm) at different concentrations (10, 30 and 50%).

Bacterial species	Water extract conc.			Mean ±SD
	10%	30%	50%	
Escherichia coli	12	16	20	16.00±4.0
Proteus mirabilis	-	7	10	5.67±5.13
Salmonella typhimurium	-	10	17	9.00±8.54
Salmonella entrica	-	6	10	5.33±5.03
Shigella dysenteriae	-	10	15	8.33±7.64
Bacillus cereus	12	15	17	14.67±2.52
Bacillus licheniformis	10	12	15	12.33±2.51
Staphylococcus aureus	17	20	24	20.33±3.51
Mean ±SD	6.38±7.09	12.00±4.75	16.0±4.72	11.46±6.72
P-value	0.009**			

^{*}P <0.05 (Significant), **P <0.01 (Highly significant), (-) no inhibition



Bacterial species

Fig. (2). Mean inhibition zone diameter of each bacterial species at three concentrations (10, 30, 50%) for Water extract of *Hyphaen ethebaica*.

Table (3) and Figures (3 & 4) showed that antibacterial activity of natural extract of *P. peruvianae* was better

than synthetic antibiotic for *Staphylococcus aureus*, and it has the same effect for *Escherichia coli*. Also, natural

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extract of *Hyphaene thebaica* was better *Staphylococcus aureus*, and it has nearly than synthetic antibiotic for the same effect for *Escherichia coli*.

Table (3): Comparison of antibacterial activities as inhibition zone diameter (mm) of natural extracts (at 50% concentration) versus standard synthetic antibiotic (Ciprofloxacin)

(elpronomenn)	Natural extracts		
Bacterial strains			Ciprofloxacin
	Ethanolic extract of	Water extract of	
	physalis peruvianae	Hyphaene thebaica	
Escherichia coli	27	20	27
Proteus mirabilis	12	10	27
Salmonella typhimurium	18	18	30
Salmonella entrica	9	10	31
Shigella dysenteriae	18	15	29
Bacillus cereus	20	17	25
Bacillus licheniformis	17	15	25
Staphylococcus aureus	23	24	21
$Mean \pm S.D$	16.12 ± 4.7	18.63 ±5.2	26.9±3.22
p-value	0.00	0.002	

*P < 0.05 (Significant), **P < 0.01 (Highly significant), (-) no inhibition

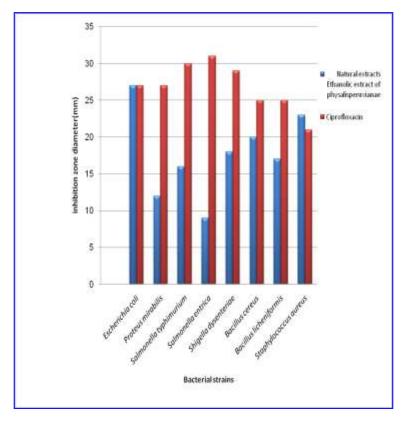


Fig. (3). Mean inhibition zone diameter of each bacterial species at ethanolic extract of *Physalis peruvianae* and ciprofloxacin.

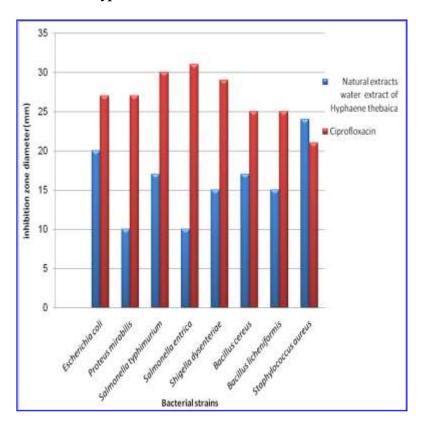


Fig. (4). Mean inhibition zone diameter of each bacterial species at water extract of Hyphaene thebaica and ciprofloxacin

Table (4). Bacteriostatic and bacteriocidal activities expressed as MICs and MBCs of Ethanol extact of Physalis neruvianae.

Bacterial Species	MIC	MBC	Ratio MBC/MIC
E.coli	12.5	12.5	=1
Proteus mirabilis	12.5	25	>1
Salmonella typhimurium	25	50	>1
Salmonella entrica	12.5	25	>1
Shigella dysenteriae	12.5	25	>1
Bacillus cereus	12.5	12.5	=1
Bacillus licheniformis	12.5	25	>1
Staphylococcus aureus	12.5	6.5	<1

MIC : Minimal inhibitory concentration (mg/ml extract) MBC: Minimal bactericidal concentration (mg/ml extract)

If MBC/MIC < or = 1 (bacteriocidal)

If MBC/MIC> 1 (bacteriostatic)

Table (5). Bacteriostatic and bacteriocidal activities expressed as MICs and MBCs of

water extact of Hyphaene thebaica.

Bacterial Species	MIC	MBC	Ratio MBC/MIC
E.coli	25	25	=1
Proteus mirabilis	25	50	>1
Salmonella typhimurium	25	25	=1
Salmonella entrica	6.5	12.5	>1
Shigelladysenteriae	6.5	12.5	>1
Bacillus cereus	6.5	25	>1
Bacillus licheniformis	12.5	25	>1
Staphylococcus aureus	6.5	6.5	=1

MIC : Minimal inhibitory concentration (mg/ml extract)

MBC : Minimal bactericidal concentration (mg/ml extract)

If MBC/MIC < or = 1 (bacteriocidal)

If MBC/MIC> 1 (bacteriostatic)

Antioxidant activity

Table (6) and Figure (5) showed that DPPH scavenging activity of Ascorbic acid (natural antioxidant) which used as

reference standard (control) increased with concentration, IC50=14.2 µg/ml (IC50: The half maximal inhibitory concentration).

Table (6). DPPH scavenging activity of different concentrations of Ascorbic acid as a reference standard.

Standard concentration (µg/ml)	DPPH scavenging activity (%)
0	0
5	11.78
10	17.49
15	54.86
20	70.49
25	77.41
30	80.65
35	87.53
40	92.48

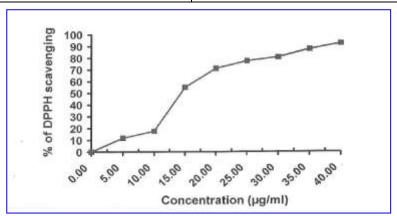


Fig. (5). DPPH scavenging activity of different concentrations of Ascorbic acid as a reference standard.

It was obvious from Table (7) and Figures (6 & 7) that the scavenging activity of both extracts from *P. peruvianae* and *H. thebaica* on DPPH

radicals increased with concentration, when: IC50 of *P. peruvianae* extract =1322 µg/ml; IC50 of *H. thebaic*a extract=771 µg/ml.

Table (7): DPPH scavenging activity of different concentrations of *Physalis peruvianae* and *Hyphaene thebaica*

Sample concentration	DPPH scavenging activity	DPPH scavenging activity (%)
(μg/ml)	(%) of physalis peruvianae	of Hyphaene thebaica
0	0	0
50	5.67	10.42
100	16.00	20.67
200	20.58	28.50
400	26.58	43.50
800	34.92	50.50
1600	58.00	75.08
5200	75.25	85.08

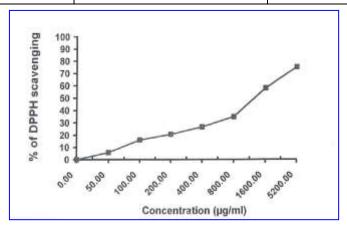


Fig. (6). DPPH scavenging activity of different concentrations of ethanol extract of *Physalis peruvianae*

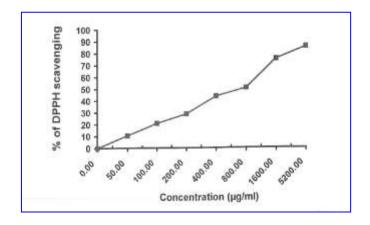


Fig. (7). DPPH scavenging activity of different concentrations of water extract of *Hyphaene thebaic*a

DISCUSSION

The market of health and herbal neutraceuticals are addressing attention to rich plants sources offering functional efficacy (Dua et al., 2013). The present results with Ethanol (70%) extract of Physalis peruvianae justified that it displays a broad antibacterial spectra and all pathogens were susceptible to it at different concentrations, except Salmonella entrica at 10 and 30% concentrations and Proteus mirabilis at 10% concentration. On the other hand, the gram-negative pathogens were sensitive to Ethanol (70%) extract ranging from 8 to 27 mm inhibition zones and the most affected Escherichia coliat was concentration with the highest mean value (19.00), while the gram-positive pathogens showed its sensitivity to Ethanol (70%) extract ranging from 10 to 23 mm inhibition zones and the most affected one Staphylococcus aureus at was concentration with mean value (17.67). This was agreed with the results of Çakir et al. (2014) who reported that Ethanol extract inhibited both gram-positive and gram-negative bacteria growth but there was more inhibition on gram-positive strains and Staphylococcus aureus was the most susceptible pathogen. Göztok and Zengin (2013) reported that the most susceptible gram-negative pathogens were proteus vulgaris followed by Escherichia coli and the most resistant pathogens were Pseudomonas aeruginosa Staphylococcus aureus.

Using water extract of Hyphaene thebaica demonstrated that all pathogens were sensitive and Staphylococcus aureus was the most sensitive strain to water extract by having maximum mean value (20.33) followed by Escherichia coli, Bacillus cereus, Bacillus licheniformis, Salmonella typhimurium, Shigella dysenteriae, Proteus mirabilis and Salmonella entrica with mean values (16.00, 14.67, 12.33, 9.00, 8.33,5.67,5.33), respectively, and there was

significant difference between different concentrations (10,30,50%) with pvalue (0.009). This result was in agreement with that of Auwal et al. (2013) who reported that aqueous extract of Hyphaene thebaica exhibited activity on both grampositive and gram-negative pathogens such as Staphylococcu saureus, Streptococcus pyogenes, Salmonella typhi, E. coli and Shigella dysenteriae and the effect of this extract on both Gram positive and negative bacteria indicated a broad spectrum antibacterial activity. Ramadan et al. (2015) and Gautam et al. (2015) reported that ethanol extract was better than hexane extract because it has higher total phenolics content and total flavonoid compounds than does hexane extract and there is a relationship between phenolic and flavonoid contents and antibacterial and antioxidant activity. Also, using ethanol as extraction solvent was better than methnol because it is less toxic than methanol, being generally recognized as a safe solvent being more suitable for further application of golden berry extract in food (Kumari et al., 2017; Codevilla et al. 2018).

Hassan et al.(2018) reported that aqueous extract of Doum fruit has greatly antibacterial and antioxidant activities because of its luxurious amounts of water soluble phenolic contents. The present results of the comparison between each extract and synthetic antibiotic (Ciprofloxacin) revealed that Ethanol (70%) extract of *Physalis peruvianae* had antibacterial activity better than synthetic antibiotic for Staphylococcus aureus, and it has the same effect for Escherichia coli. Water extract of Hyphaene thebaica had antibacterial activity better than synthetic antibiotic for Staphylococcus aureus, and it has nearly effect for Escherichia coli. This was in agreement with Ömer et al. (2017) who found that ethanolic extract of Physalis peruvianae fruit was more effective than ampicillin and cephazolin against S. aureus.

Auwal et al. (2013) reported that aqueous extract of Hyphaene thebaica had nearly effect to tetracycline. The MIC index or (MBC/MIC ratio) was calculated for each of the investigated extracts and Rakholiya et al. (2013) and karou et al. (2005) reported that if the ratio is greater than 1, it is considered as microbiostatic, while with ratio equal or smaller than 1 it is considered as microbiocidal. The present results indicated that ethanolic extract of physalis peruvianae has bacteriocidal effect on three pathogenic strains (E.coli, Bacillus cereus and Staphylococcus aureus) and bacteriostatic effect on the other pathogens. Water extract Hyphaene thebaica has bacteriocidal effect on three pathogenic strains (E. coli, Salmonella typhimurium Staphylococcus aureus) and bacteriostatic effect the other pathogens. Abootalebian et al. (2016) reported that there is strong correlation and positive relationship between polyphenols concentration and antimicrobial and antioxidant activities in plant extracts.

In the current work, the ethanolic extract of Physalis peruvianae and water Hyphaene thebaica have extract of significant antioxidant activity (scavenging against **DPPH** radicals) comparison with standard antioxidant (ascorbic acid). Ramadan et al. (2015) reported that ethanolic extract of physalis peruvianae has higher radical scavenging activity than hexan extract. DO et al. (2014) and Codevilla et al. (2018) reported that the antioxidant capacity of Physalis peruvianae fruit extract obtained with ethanol was higher than methanol. Chang (2009)found that when al.extract increased, the concentration of DPPH radical scavenging activity increased.

El-Beltagi *et al.* (2018) recorded that doum fruit aqueous extract contain high amount of flavonoids, phenols and it was used as antioxidant and antibacterial material which can alleviate the adverse

effects of oxidative stress and prevent diseases caused by pathogenic bacteria and indicated that doum fruit extracts are highly effective free radical scavengers. Doum fruit extract possesses significant antioxidant and antimicrobial activities (Mohammed *et al* 2018). Laith *et al*. (2018) found that antioxidant activity of doum fruit extracts increased by increasing the concentrations of its extracts.

In conclusion, side effects resulted from using synthetic antibiotics and chemical preservatives can be avoided by replacing them by natural plant extract as ethanolic extract of *Physalis peruvianae* and water extract of *Hyphaene thebaica*. So, further investigations on large scale are recommended.

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النشاطات المضادة للبكتيريا والمضادة للأكسدة لمستخلصات نباتى: الحرنكش (فيساليس بيروفيانا) والدوم (هيفين تيبيكا)

سارة مختار عبد المجيد*1 ، هالة محمد أبوشادى*2 ، عفاف على أمين*1 . 1 المعهد القومى للتغذية – قسم صحة الطعام – وحدة الميكر وبيولوجى – مصر 2 جامعة عين شمس – كلية العلوم – قسم الميكر وبيولوجى - مصر

المستخلص

لقد تم اختبار التأثيرات المثبطة للبكتيريا لكلا من المستخلص الايثانولي لنبات الحرنكش (فيساليس بيروفيانا) والمستخلص المائي لنبات الدوم (هيفين ثيبيكا) على ثمان سلالات من البكتيريا (ايشيريشيا كولاي، بروتيس ميرابيلس، سالمونيلا تايفيميوريم، سالمونيلا انتريكا، شيجلا ديسينتريا، باسيلاس سيرس، باسيلاس ليشنيفورميس، استافيلوكوكاس اورياس) كان استافيلوكوكاس اورياس) كان

استافيلو تحوكاس اورياس) باستخدام طريقه الانتشار. وقد نبين من هذه الدراسة أن ميكروب (استافيلو توكاس اورياس) كان اكثر السلالات موجبة الجرام تاثرا ، وميكروب (ايشيريشيا كولاي) هو اكثر السلالات سالبة الجرام تاثرا لمستخلص الايثانولي لنبات الدوم - هيفين ثيبيكا. وعند مقارنة النتائج كانت مقاربة لنتائج المضاد الحيوى الاصطناعي (السيبروفلوكساسين).

كما تم اختبار النشاط المضاد للاكسدة لكل من المستخلصين عن طريق السيطرة على الشوارد الحرة المعلومة مسبقا (2 - 2 - ثنائي فينيل ، 1- بيكريل هيدرازيل) و استخدام حمض الاسكوربيك كمرجع. وقد اظهرت النتائج أن قدرة كلا من المستخلصين في التحكم على هذه الشوارد الحرة تزداد بزيادة تركيز كلا منهما. لذا المستخلص الايثانولي لنبات الحرنكش (فيساليس بيروفيانا) والمستخلص المائي لنبات الدوم (هيفين تيبيكا) يعدا مصدرا واعدا كمضادا ميكروبي طبيعي ، كمضادا للاكسدة و كمواد حافظة طبيعية للأغذية المصنعة .