EFFECT OF SILYMARIN AS NATURAL ANTIOXIDANTS AND ANTIMICROBIAL ACTIVITY

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Abstract

Effect of silymarin as natural antioxidants and antimicrobial activity was main target of the present study. Physicochemical properties of the sunflower oil, and its fatty acid fractionated using GLC method were determined. The ratio between ∑ PUFA / ∑SFA, MUFA / ∑PUFA and ∑USFA / ∑SFA was 4.23, 0.49 and 6.29 respectively. Antioxidant activities of the silymarin were evaluated using Rancimat test at 100 ºC and calculated at 25ºC using the temperature coefficient of 2.2 and the DPPH• radical scavenging method. The addition of the different concentrations of silymarin to sunflower oil (100 to 500ppm) led to clear increment in relative stability % and increasing index of sunflower oil (137.589 to 144.889% and 37.563 to 44.880) compared with BHT (107.329% and 7.317) at100ºC respectively. The DPPH• radical scavenging activities (%) were increased with increasing silymarin concentration from 100 to 450 ppm from70.27±0.19% to 86.77± 0.0.2% respectively. Meanwhile 500ppm of silymarin slightly lowered its value to 83.78±0.15% than that of 300 to 450ppm (86.77±0.02%) still higher scavenging activity (%) compared with BHT (72.78±0.25%) at 200ppm. Antimicrobial activities of each concentration of silymarin were measured and the data indicated that different concentrations of silymarin had exhibited antimicrobial activities against gram-positive bacteria (*Bacillus subtilis* ATCC 33221,*Bacillus cereus* ATCC 33018,*Bacillus cereus* NRRLB 3711and *Staphylococcus aureus* ATCC 20231), gram negative bacteria (*Escherichia coli* ATCC69337, *Escherichia coli* 0157 and *Pseudomonas aeruginosa*, ATCC 9027) , molds (*Aspergillus niger* NRRL326 , *Aspergillus flavus* MERCN 101, *Aspergillus parasiticus* and *Penicillium sp.*) and yeast (*Geotericum candidum* NRRLY552). No antimicrobial activity of silymarin concentrations was noticed against either *Saccharomyces cerevisiae* NRRLY 3034 or *Candida lipolytica* NRRLY1095. The antimicrobial activity of silymarin was found to be increased in parallel with increasing the concentrations against all tested microorganisms strains. It could be concluded that, the silymarin can be used as natural antioxidants and antimicrobial activity in food industry.

Key word: Silymarin, natural antioxidants, antimicrobial activity, Rancimat DPPH , BHT, Sunflower oil.

INDTRODCTION

Silymarin is a lipophilic extract from the seeds of milk thistle and is composed of three isomer flavonolignans, silybin, silydianin and silychristin. Silymarin content of milk thistle seed ranged from 4 to 6% based on dry weight. Silybin is the component with the greatest degree of biological activity and comprises 50–70% of silymarin (Ramasamy and Agarwal, 2008). Silymarin has free radical scavenging properties and its ability to enhance endogenous anti-oxidant defense systems in vivo . Silymarin has been shown to inhibit the growth of human prostate, breast ، and cervical cancer cells in test tubes. Silymarin can protect liver, brain, heart and other vital intra organs from oxidative damage for its ability to prevent lipid peroxidation and replenishing the reduced glutathione levels. (Das *et al.,* 2008). silymarin have anti-inflammatory and anti-arthritic effects due to excellent antioxidant property, scavenging free radicals which act as pro-inflammatory agents. Silymarin was found to be more effective in cases of developing arthritis compared to developed arthritis. Silymarin and silibinin hinder inflammatory process by inhibiting neutrophil migration and kuppfer cell inhibition. They also inhibit the formation of inflammatory mediators viz. prostaglandins and leukotriens and release of histamine from basophils(Dixit *et al.,* 2009). Silymarin is a strong antioxidant that has been proven to promote liver cell regeneration, to reduce blood cholesterol and to help prevent cancer (Vaknin *et al.,* 2008).

The hepatoprotective action of silymarin is mainly through its anti-free radical and anticarcinogenic roles (Shaker *et al.,* 2010), but it has also been ascribed other actions includes antioxidant, anti-lipid peroxidative, antifibrotic, anti-inflammatory immunomodulatory and liver regenerating activity. Silymarin has clinical applications in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug diseases of the liver, psoriasis and in neuroprotective and neurotropic activity (Ghosh *et al.,* 2010).Silymarin acts as a toxin blockade agent by inhibiting binding of toxins to hepatocyte cell membrane receptors .The toxicity of silymarin is very low, the oral 50% lethal dose being 10,000 mg kg^{-1} in rats and the maximum tolerated dose being 300 mg kg^{-1} in dogs (Abenavoli *et al.,* 2010).Silymarin is not soluble in water and is usually administered in capsules as a standard extract (70–80% silymarin). Formulation of the phytotherapic extract (silymarin) in self-emulsifying pellets gave rise to a simultaneous improvement of the oral biodiversity of its main active compounds (Iosio *et al.,* 2010). Silymarin has been shown to reduce liver fibrosis up to 30–35%, and in few cases it has reversed the liver fibrosis (Haddad

etal., 2011). Silymarin has potent antioxidant properties, reduces and suppress harmful effects of solar ultraviolet radiation (UVR) (Altaei 2012). Silymarin is well tolerated in chronic HCV-infected patients. However, no evidence of salutary effects of oral silymarin has yet been reported based on intermediate endpoints (ALT and HCV RNA) in this population. Moreover, intravenous administration of silymarin should be further studied.(Zongguo *et al.,* 2014).Silymarin have the potential to protect the cell from genotoxic damage, due to a lower value DNA in tail. Effective prevention against ROS production. Katerina *et al*., (2014). Silymarin cream was a novel, effective and safe treatment modality for melasma especially inepidermal and mixed types in Fitzpatrick skin phototype III, IV and V as it showed a significant improvement of melasma lesion. It was as effective as 50% glycolic acid peeling in the treatment of melasma without postinflammatory hyperpigmentation that occurred by glycolic acid peeling. (Elfar and El-Maghraby 2015). Silymarin combined with grape seed extract had synergistic effect as antifibrotic therapy than single treatment with silymarin or GSE alone and GSE (200 mg/Kg) combined with silymarin had a good resultant effect. Nada *et al.,*(2015). Seeds of milk thistle contain small amounts of flavonoids (taxifolin) and approximately 20–35% fatty acids and other polyphenolic compounds (Ramasamy and Agarwal, 2008). A number of other flavonolignans have also been found in the seeds including isosilybin, dehydrosilybin, desoxysilycristin, desoxysilydianin, silandrin, silybinome, silyhermin and neosilyhermin (Katerina *et al*. (2014). Therefore, milk thistle seed may possess anti allergic and anti-asthmatic activities (Dixit *et al.,* 2009). Further in vivo studies are needed to determine whether milk thistle is safe or effective for people with forms of cancer(Das *et al.,* 2008).

The main objective of the present study was to investigate effect of silymarin as natural antioxidants and antimicrobial activity substance.

MATERIALS AND METHODS

Materials

Sunflower oil Refined bleached and deodorized sunflower oil (with no added antioxidants) was obtained from Cairo for Oils and Soap Company, Giza, Egypt. All the chemicals and reagents used were of analytical reagent grade and were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany) Company. Silymarin were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany) Butylated hydroxyl toluene (BHT):

BHT and 2, 2-diphenyl-1 picryl hydrazil (DPPH) was purchased from Sigma Chemical Co. (St. Louis, Mo 63178, USA).

Microbiological evaluation

Microorganisms : some bacterial strains representing gram–negative bacteria *Escherichia coli* ATCC 69337 , *Escherichia coli* 0157, *Pseudomonas aeruginosa* ATCC 9027, and gram-positive bacteria *Bacillus subtilis* ATCC 33221, *Bacillus cereus* ATCC 33018 , *Bacillus cereus* NRRLB 3711 and *Staphylococcus aureus ATCC 20231* in addition to 3 strains of yeasts *Saccharomyces cerevisiae* NRRLY 2034, *Candida lipolytica* NRRLY 1095 , *Geotericum candidum* NRRLY 552 , in addition to 3 strains of moulds *Aspergillus flavus* 101 MERCN , *Aspergillus niger* NRRL 326, *Aspergillus parasiticus* and *Penicillium sp* were obtained from MERCN Cairo (Microbial Collection Center, the Faculty of Agriculture , Ain Shams University) . These microorganisms were checked for their purities likewise they were reactivated to obtain active microorganisms.

Used media

Nutrient agar medium. The medium was obtained from El Nasr Pharmaceutical Chemical Co., Egypt and Malt agar medium which used for yeasts and moulds growth was obtained from Biolifes, Milano, Italy

Methods

Physicochemical properties of the Sunflower oil**:**

The peroxide value, acid value, refractive was determined according to the method described in the A.O.A.C (2005).

Determination of fatty acids profile

Fatty acid profile of sunflower oil were esterified into their corresponding FAMEs using methanoleic NaOH and BF3 with methanolic (Boron triflouride) as described by A.O.A.C. (2005). Evaluation of antioxidant properties of Silymarin on sunflower oil

Determination of antioxidant activity of silymarin by Rancimat test

Sunflower oil (5g), was mixed separately with different amounts of silymarin 0 , 100, 150,250,300, 350, 400,450 and 500 silymarin and 200 ppm of BHT antioxidants and then exposed to the rancimat test using Metrohm Rancimat model 734 at 100ºC and calculated at 25ºC using the temperature coefficient of 2.2 for induction period at an airflow of 20 L/ h. Measuring vessels, electrodes, connecting tubes and glassware were cleaned several times before the experiments (Farhoosh and Tavassoli-Kafrani 2011). The relative stability (%) and the increasing index were calculated using the following equations:

$$\text{Relative stability (\%)} = \frac{\text{Induction period of sample} \times 100}{\text{Induction period of control}}$$

$$\text{Increasing index} = \frac{\text{Induction period of sample} - \text{Induction period of control} \times 100}{\text{Induction period of control}}$$

Determination of antioxidant activity of silymarin by (DPPH$^{\bullet}$ **test)**

The free radical scavenging activity of different concentration of silymarin were measured by the 2, 2-diphenyl-1 picryl hydrazil (DPPH$^{\bullet}$) method according to (Kekuda *et al.,* 2010). The scavenging activity was calculated using the folwwing formula:

DPPH scavenging activity % = (A blank –A sample / A blank) x 100

Antimicrobial activity of Silymarin

The effect of Silymarin on bacterial and yeast growth was studied by the Paper-Disc plate method according to Jiang *et al.,* (*2012*) by measuring the inhibition zone in mm .

RESULTS AND DISCUSSION

Physical and chemical properties of sunflower oil

The physical and chemical properties of sunflower oil used in the present study were determined to define the identity of the oil, and insure that oxidative reaction has not begun. The obtained results are shown in Table (1). The acid values was 188 mg KOH/g oil where the low acid values (188-194 mg KOH/g oil) of the oil indicated its freshness state. Moreover, the physical and chemical properties of sunflower oil under study were found to be corresponding to the values approved by the Egyptian Standard (49- 1993), for sunflower seed oil (refractive index: 1.4681, acidity (0.05 as % oleic acid), peroxide value 0.0 equivalent of active oxygen / kg. of oil, iodine value: 118, saponification value: 178, and unsaponifiable matter % not more than 1.5%). The above mentioned data revealed that no lipolysis and /or oxidative rancidity occurred in the oil under study. Therefore, the oil can be used in study and the previous obtained data were in harmony with the findings of (Firestone, 2005) , Hilali *et al.,* (2005) and Yunus *et al.,* 2009).

Table 1. Physicochemical properties of the Sunflower oil.

Parameter	Physiochemical properties of sunflower
Odor	Acceptance
Color	Yellow
Refractive index	1.4681
Acidity (as % oleic acid)	0.05
Acid value (mg KOH/g oil)	188
Iodine value	118
Peroxide value (meq O/kg oil)	0.00
Unsaponifiable matter(g/kg)	1.5
Saponification value (mg /g oil)	178
FFA %(as Oleic acid)	0.11

Fatty acids composition of sunflower oils

The fatty acids composition of sunflower oil show in **Table (2).** The results indicated that sunflower oil contains five saturated fatty acids, i.e., merestic (14:0), palmetic (16:0) , stearic acid (18:0) and arachidonic acid (C 20:0) in relative percentages of 0.13%, 8.03% ,4.32% and 0.96% respectively, comprising 13.67% of Saturated fatty acids (SFA) . Moreover, there monounsaturated fatty acids (MUFA), i.e.(16:1),(18:1) and(20:1) showed relative percentages of 0.20%,27.4 % and 0.48% resp., comprising 28.08% of Monounsaturated fatty acids (MUFA) . Linoleic acid (C18:2) and α-Linolenic acid (C18:3) as resulted in a value of 55.1% and 2.79%, respectively, comprising 57.89 of Polyunsaturated fatty acids (PUFA) .

In sunflower oil, the main fatty acid is Linoleic acid, followed by oleic, Palmitic and Stearic acids. Also, these results were confirmed by the ratio between ∑ PUFA / ∑SFA, MUFA / ∑PUFA and ∑USFA / ∑SFA ,(4.234 , 0.485 and 6.289) respectively which showed that the oil had a high TU/ TS ratio (6.289). This means that sunflower oil is distinguished by containing fatty acids profile with high degree of unsaturation such as $C_{18:2}$ (essential fatty acid) Moreover, the obtained data was in harmony with the findings **Table(2)** the fatty acid composition of sunflower oil . These results are agreement with Firestone (2005), Hilali *et al.,* (2005) and Yunus *et al.,* 2009).

Ta b l e 2. Fatty acids composition of sunflower oils

Fatty acids (%)	Sunflower oil
Myristic acid (C14:0)	**0.13**
Palmitic acid (C16:0)	8.03
Arachidonic acid C 20:0	0.23
Stearic acid (C18:0)	4.32
Arachidic acid (C22:0)	0.96
∑Saturated fatty acids (SFA)	13.67
(C16:1)	0.20
Oleic acid (C18:1)	27.4
Gadoleic acid (C20:1)	0.48
∑Monounsaturated fatty acids (MUFA)	28.08
Linoleic acid (C18:2)	55.1
α-Linolenic acid (C18:3)	2.79
∑Polyunsaturated fatty acids (PUFA)	57.89
Others	0.36
∑PUFA /∑ SFA	4.23
∑MUFA / ∑PUFA	0.49
∑USFA / ∑SFA	6.29
C18:2 / C18:3	19.75

Effect of different concentrations of the silymarin on oxidative stability of sunflower oil

The antioxidant properties of the silymarin on refined sunflower oil were determined by Rancimat test at 100 ºC and calculated at 25ºC using the temperature coefficient of 2.2 .The obtained results are recorded in **Tables (3)** . From these results, it could be found that addition of silymarin exhibited antioxidant activities of fresh sunflower oil compared with control sample.

The data in **Table (3**) showed that silymarin at level 100 to500 ppm had highly antioxidant activity more than that of BHT at 200 ppm or control sample. The induction period of sunflower oil in the presence of 100 to 500 ppm silymarin increased the induction period to about 13.649 - 14.375 hr compared with that of 9.922 and 10.648 hr for control and BHT respectively.

Relative stability (%)and induction index (table 3) in parallel with those of induction period, for instance, the relative stability increased gradually from 137.56 to 145.36% due to silymarin concentration from 100- 450 ppm while a slight decrease was found due to 500ppm silymarin (144.88%) compared with BHT

(107.32 %). Similar trend was found concerning increasing index which gradually increased from 37.56 to 45.36 due to 100-450 ppm silymarin concentration with slight decrease due to 500 ppm (44.88) compared with BHT (7.31)

The calculated induction period related in 5-8 months as a shelf life (25C) as a result of silymarin concentration.

Finally, from these data, it could be concluded that these results of the antioxidant properties of the silymarin on refined sunflower oil were determined by Rancimat test at 100 ºC and calculated at 25ºC using the temperature coefficient of 2.2 are in agreement with those observed by antioxidant activity of silymarin by DPPH test .

Table 3. Effect of different concentrations of the silymarin on oxidative stability of sunflower oil by Rancimat test at 100 ºC.

Treatment	Induction period		Relative stability (%)		Increasing index	
	100 ºC(hr)	25 ºC (months)	100 ºC	25 ºC	100 ºC	25 ºC
Control (0ppm)	9.922	5.499	100	100	0	0
B.H.T (200ppm)	10.648	5.902	107.329	107.329	7.317	7.329
Silymarin (100ppm)	13.649	7.566	137.563	137.589	37.563	37.589
Silymarin (150ppm)	13.697	7.592	138.047	138.061	38.047	38.061
Silymarin (200ppm)	13.794	7.646	139.024	139.043	39.024	39.043
Silymarin (250ppm)	14.326	7.941	144.386	144.408	44.386	44.408
Silymarin (300ppm)	14.423	7.995	145.364	145.39	45.364	45.39
Silymarin (350ppm)	14.423	7.995	145.364	145.39	45.364	45.39
Silymarin (400ppm)	14.423	7.995	145.364	145.39	45.364	45.39
Silymarin (450ppm)	14.423	7.995	145.364	145.39	45.364	45.39
Silymarin (500ppm)	14.375	7.968	144.880	144.899	44.880	44.899

Antioxidant activity of silymarin by (DPPH$^{\bullet}$ **test)**

The DPPH$^{\bullet}$**scavenging activity (%) of the silymarin**

The Free radical–scavenging capacities of silymarin compared with Butylated hydroxytoluene (BHT) at 200 ppm were measured by DPPH assay at different concentrations are given in **Table (4).** The DPPH radical scavenging activities (%) were increased with increasing silymarin concentration from 100 to 450 ppm in a range of 70.27±0.19% to 86.77±0.02% respectively. A high antioxidant activity was found for silymarin (86.77±0.02%) at 300 to 450 ppm compared with BHT (72.78±0.25%) at 200 ppm. The highest concentration of silymarin (500ppm)

resulted in lower value (83.78±0.15%) than concentration 300 to 450ppm (86.77±0.02%) but were also it had higher scavenging activity (%) compared BHT (72.78±0.025%) at 200ppm.

On the contrary, no remarkable radical–scavenging capacities had noted for silymarin at 300 to 450 ppm (86.77±0.02%). meanwhile 500ppm concentration slightly decrease of the DPPH scavenging activity to 83.78±0.15%.

From the above-mentioned data the DPPH• scavenging activity (%)of silymarin are in agreement with those found by Rancimat test at 100 ºC and calculated at 25ºC

Table 4. DPPH scavenging activity (%) of the silymarin.

Treatments	DPPH• **scavenging activity (%)**
Butylated hydroxytoluene (BHT) 200ppm	72.78±0.25
Silymarin (100ppm)	70.27±0.19
Silymarin(150ppm)	73.71±0.11
Silymarin (200ppm)	77.89±0.20
Silymarin (250ppm)	82.80±0.01
Silymarin (300ppm)	86.77±0.02
Silymarin (350ppm)	86.77±0.02
Silymarin (400ppm)	86.77±0.02
Silymarin (450ppm)	86.77±0.02
Silymarin (500ppm)	83.78±0.15

It could be concluded that the silymarin considered as good natural antioxidant which prolongs stability of sunflower oil and it may be a potent antioxidant activity for its stabilization

Evaluation of antimicrobial activity of silymarin .

The antimicrobial activities of different concentrations of silymarin were determined against seven bacterial two yeast and four mold strains. The different concentrations of silymarin that used were 100, 150, 200, 250, 300, 350, 400, 450 and 500ppm . The diameter of inhibition zones was taken as a criterion of the antimicrobial spectrum. The obtained results are presented in **Table (5).** Data indicated that the different concentrations of silymarin exhibited antimicrobial activities against gram-positive bacteria (*Bacillus subtilis ATCC 33221*, *Bacillus cereus ATCC 33018,Bacillus cereus NRRLB 3711and Staphylococcus aureus ATCC 20231*), gram negative bacteria (*Escherichia coli ATCC69337, Escherichia coli 0157 and Pseudomonas aeruginosa, ATCC 9027) , molds (Aspergillus niger NRRL326 ,Aspergillus flavus* MERCN *101, Aspergillus parasiticus* and *Penicillium sp.*) and yeast (*Geotericum candidum NRRLY552*) . No antimicrobial activity of the different

concentrations of silymarin was noticed against either Saccharomyces cerevisiae NRRLY 3034 or Candida lipolytica NRRLY1095 while different concentrations of silymarin exhibited antimicrobial the concentrations against all tested microorganisms strains. The antimicrobial activity activity against Geotericum candidum NRRLY 552 (21- 26mm inhibition zone). The antimicrobial activity of the different concentrations of silymarin was increased with increaseing of the different concentrations of silymarin and showed higher a effective on molds compared with that all tested bacterial strains (gram positive or negative gram).

In general, data in **Table (5)** , indicated that different concentrations of silymarin had the most powerful antimicrobial and molds spectra against all tested microorganisms antimicrobial spectra (318.9, 331.9, 313.9, 352.5, 362.2, 369.5, 385.7, 392.2,and 402.3) which in parallel with the concentrations of silymarin from 100 to 500 ppm respectively

Table 5. Antimicrobial activity of silymarin

Organisms	**Diameters of inhibition zones (mm)* of silymarin concentrations(ppm)**								
	100	150	200	250	300	350	400	450	500
Gram positive bacteria									
Bacillus subtilis ATCC 33221	23	23.5	24.5	25	26	26.2	26.5	27.5	28
Bacillus cereus ATCC 33018	22	23	23.5	24.5	25	26	26.2	26.5	27.5
Bacillus cereus NRRLB 3711	22	23.2	23.7	24.7	25.2	26.3	26.5	26.8	27.6
Staphylococcus aureus ATCC 20231	21	22	23	23.5	24	24.7	25.6	26	27
Gram negative bacteria									
Escherichia coli ATCC69337	25	25.4	26	26.5	27.1	27.3	28	28.4	29
Escherichia coli 0157	25.1	25.5	26.2	26.5	27.2	27.5	28	28.4	29
Pseudomonas aeruginosa ATCC 9027	24.8	25.3	26	26.3	26.5	27	27.5	28.4	29.1
Yeast									
Saccharomyces cerevisiae NRRLY2034	0	0	0	0	0	0	0	0	0
Candida lipolyticaNRRLY1095	0	0	0	0	0	0	0	0	0
Geotericum candidum NRRLY552	21	21	21.5	22	23	23	24	24.5	26
Molds									
Aspergillus niger NRRL326	35	37	40	42	44	45.3	46	46.5	47
Aspergillus flavus MERCN 101	37	41	43	44	45.5	46	46.5	47	48
Aspergillus parasiticus	32	33	3.5	34	34.6	34.7	44.4	45.2	46.2
Penicillium sp.	31	32	33	33.5	34.1	35.5	36.5	37	37.9
Total antimicrobial spectra	318.9	331.9	313.9	352.5	362.2	369.5	385.7	392.2	402.3

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تأثير السليمارين كمضاد أكسدة طبيعى ومضاد لنمو الأحياء الدقيقة

عبد العظيم سيد عبد العظيم عبد اللطيف

معهد بحوث تكنولوجيا الاغذية – مركز البحوث الزراعية

اجري هذا البحث لدراسة مدى أمكانية استخدام السليمارين كمضاد أكسدة ومضاد لنمو الأحياء الدقيقة كما تم تقدير الخصائص الفيزوكيمائية لزيت دوار الشمس كانت الاحماض الدهنية كلا من اللينوليك و واوليك وبالمتيك واستيارك ايضا كانت نسبه مجموع (الاحماض الدهنية عديدة عدم التشبع / الاحماض الدهنية المشبعة ، الاحماض الدهنية احادية عدم التشبع / الاحماض الدهنية عديدة عدم التشبع و الاحماض الدهنية غير المشبعة / الاحماض الدهنية المشبعة (٤,٢٣٤ ، ٠,٤٨٥ و٦,٢٨٩) على التوالى . وتم تقييم النشاط المضاد للاكسدة باستخدام الرانسيمات على درجة حرارة ١٠٠ م و حساب درجة الحرارة على ٢٥ م باستخدام معامل تصحيح ٢,٢ و طريقة DPPH• . اضافة تركيزات مختلفة من ١٠٠ الى ٥٠٠ جزء فى المليون ادى الى زيادة واضحة فى نسبة درجة الثبات ومعدل الزيادة لزيت دوار الشمس (١٣٧,٥٨٩ الى ١٤٤,٨٨٩% و ٣٧,٥٦٣ الى ٤٤,٨٨٠) مقارنة ب BHT (١٠٧,٣٢٩ % و ٧,٣١٧) عند ١٠٠م على التوالى . وجد ان النشاط المضاد للاكسدة باستخدام طريقة DPPH• يزداد بزيادة التركيز من ١٠٠ الى ٤٥٠ جزء فى المليون ٧٠,٢٧± ٠,١٩ % الى ٨٦,٧٧ ± ٠,٠٢% على الترتيب . بينما ينخفض عند تركيز ٥٠٠ جزء فى المليون (٨٣,٧٨ ±٠,١٥ %) اعلى من التركيز ٣٠٠ الى ٤٥٠ جزء فى المليون ٨٦,٧٧ ±٠,٠٢ % ولكن جميع التركيزات كان لها اعلى قيمة مقارنة ب BHT (٧٢,٧٨ ±٠,٠٢٥%) عند تركيز ٢٠٠ جزء فى المليون . كما تم قياس النشاط المضاد لنمو الاحياء الدقيقة لجميع التركيزات من السيلمارين حيث اظهرت والنتائج ان مختلف التركيزات اظهرت نشاط مضاد لنمو البكتريا الموجبة (*Bacillus subtilis* ATCC 33221, *Bacillus cereus* NRRLB 3711 و*cereus* ATCC 33018 *Staphylococcus aureus* ATCC 20231) او السالبة لجرام (*Escherichia coli* 0157 *Escherichia coli* ATCC69337, و *Pseudomonas aeruginosa*, ATCC 9027) و الفطريات (*Aspergillus niger NRRL326 ,* *Aspergillus flavus MERCN101 101,Aspergillus par* و *Penicillium sp*) و الخميرة (*Geotericum candidum* NRRLY552) . لم نلاحظ اى تاثير مضاد لنشاط خميرة الخباز(*Saccharomyces cerevisiae* NRRLY 3034 و*Candida lipolytica* NRRLY1095) . النشاط المضاد لنمو الاحياء الدقيقة يزداد بزيادة تركيز السلمارين .

لذا توصى الدراسة باستخدام السليمارين كمادة طبيعية مضادة للتاكسد ولنمو الاحياء الدقيقة فى صناعة الاغذية

الكلمات الدالة:. السيلمارين ، مضادات الاكسدة الطبيعية ، النشاط المضاد للاحياء الدقيقة ، الرنسيمات ، DPPH، BHT، زيت دوار الشمس .