Chemical composition and biological activities of flavonoids extract from safflower flower (*Carthamus tinctorius* L.)

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Abstract: The antifungal, antioxidant activities and chemical composition of total flavonoids extract from safflower flower (*Carthamus tinctorius* L.) were evaluated. Results showed that the percentage yield of total flavonoids extract of *Carthamus tinctorius* L. was 14%. The experimental data of study revealed that total flavonoids showed 88.1% antioxidant activity. The antifungal activity of total flavonoids extract at five concentrations (100,200,300,400 and 500µg/ml) was tested against eight selected phytopathogen fungi. Results indicated that *Alternaria alternate, Sclerotium rolfsii, Sclerotonia sclerotorium* and *Fusarium oxysporium* were most sensitive fungi to total flavonoids extract comparing with the other tested fungi which EC_{50} were 235.2, 280.7, 281.9 and 321.3ppm, respectively. On the other hand, *Rhizoctonia solani* was more tolerance to total flavonoids extract which EC_{50} was highest concentration (485.1ppm) comparing with the other fungi. High-performance liquid chromatography (HPLC) analysis of total flavonoids extract revealed that the safflower flower contains 17 flavonoids compounds . Hespirdin is the major compound which was (8813.28ppm) followed by quercetrin (1000.50ppm), Apig-6-arbinose-8-glactose (631.49ppm) and rutin (513.06ppm).

The study indicated that total flavonoids extract of *Carthamus tinctorius* L. has potential antioxidant and antifungal activities . These effects may be attributed to glycoside compounds.

Keywords: Total flavonoids, *Carthamus tinctorius* L., antioxidant activity , antifungal activity, phytopathogen fungi, HPLC analysis, glycoside compounds.

1.Introduction

Carthamus species probably originate from Southern Asia and is known to have been cultivated in China, India, Iran and Egypt (**Bae *et al.*, 2002**). Plant secondary metabolites represent a good of noval antimicrobial molecules. *Carthamus tinctorius* L. it belongs to Asteraceae family in the order of Asterales that contains about 22.750 genera and more than 1.620 species. The colour of flower varies from whitish yellow to red orange. Many Chinese medicines are prepared by using dried flowers and extract of flowers. The plant extracts containing pigments are used in treatment of many diseases such as menstrual problem, cardio vascular diseasespain and swelling associated with trauma. These medicinal preparation have been widely accepted which helps in increased the demand for the safflower flower (**Deodhar, 2001**).

Flavonoids are widely distributed in the plant kingdom. They constitute the largest group of polyphenol and are considered to be responsible for the color and taste of many fruits and vegetables. Flavus means yellow in Latin (**Bueno *et al.*, 2012**). A variety of biological activities have been reported for flavonoids such as antioxidant, anti-inflammatory, estrogenic, antimicrobial and antitumor abilities and different experimental approaches can be used for their evaluation (**Santos-Buelga and Felicians, 2017)**. Flavonoids are becoming the subject of antimicrobial research and many groups have isolated and identified the structures of flavonoids with antifungal, antiviral and antibacterial activities (**Estelle *et al.*, 2011**). There is an urgent need to develop new and more effective antifungal drugs because of the increased resistance of fungi to the drugs currently used in clinical practices (**Rahalison *et al.*, 1994**).

Carthamin, a flavonoid type of dye is the main contains in *C. tinctorius* flowers (**Shirwaikar *et al.*, 2010**(. Also, The flowers contains cathamidin, isocarthamidin, quercetin, kaempferol and 6-hydroxy kaempferol (**Jin *et al.*, 2008**). The flavones luteolin and its glucopyranoside, i.e. luteolin-7-o-beta-D-glucopyranoside and luteolin-7-o-(6-o-acetyl)–beta-D-glucopyranoside were found in *C. tinctorius* flowers (**Xiao and Liu, 2005**). The flowers of this plant contain acacetin, luteolin, rutoside, nicotiflorin, cynaroside and quercetin (**Yoon *et al.*, 2007 and Yue *et al.*, 2013**).

Antioxidants are defense against free radical damages and critical for maintaining optimum health. The need for antioxidants becomes even more critical with increased exposure to free radicals, which are electrically charged molecules with unpaired electrons, pollution, cigarette smoke, drugs, diseases, stress and even exercise can increase free radical exposure (**Ania *et al.*, 2012**).

So in the present study, an attempt was made to evaluate the antioxidant and antifungal activities besides the chemical composition for total flavonoids extract from safflower flower powder (*Carthamus tinctorius* L.).

2.Materials and methods

2.1. plant materials

The plant material (safflower flowers powder, *Carthamus tinctorius* L.) used in this study was obtained from the Egyptian local market.

2.2. Extraction of flavonoids

Extraction of flavonoids was made according to (**Sabah and Saleh, 2015**). Twenty five grams of defatted plant soaked in 70% ethanol for 6 hours, the extracted filtered through filter paper (Whatman No.1). Then, added 2% aqueous lead acetate until forming brown precipitate . The precipitate that produced converted to salt chloride by dissolving in (50ml acetone and 10ml 2N HCl). The precipitate separated by centrifuged under cooling (10.000rpm /15min). The precipitate placed in petri dish at room temperature until it dryness. The residue was weighted and stored at 4°C until use. The percentage yield was calculated as follows:

$$\text{Yield\%} = \frac{\text{Weight of extract recovered}}{\text{Weight of dry powder}} \text{ X } 100$$

2.3. Fungal strains

Cultures of plant pathogenic fungi (*Rhizoctonia solani, Sclerotium rolfsii, Sclerotonia sclerotorium, Fusarium solani, Fusarium oxysporium, Botrytis cinerea, Alternaria alternate* and *Humicola fuscoatra)* were provided by Fungicide, Bactericide and Nematicide Research Department, CAPL. Each fungus was maintained on potato dextrose agar (PDA) and stored at 5°C for further studies.

2.4. Antifungal assay

Antifungal activity of plant was determined by food-poisoned technique (**Mohanty *et al*., 2012**). Standard extracts at 100, 200, 300, 400 and 500µg/ml were mixed with 50ml of sterilized PDA medium and transferred equally into three Petri dishes. The media was allowed to solidify. Then seven day old fungal culture disk of 6-mm diameter was taken and inoculated to the center of Petri dishes containing plant extracts. Instead of PDA medium without plant extract served as control. All dishes were incubated at 27±2°C and radial growth of colony was measured when the mycelia of control had almost filled the Petri dishes. Each test was performed in triplicate.

The fungal growth inhibition which was calculated due to treatment against control using the following formula: **(Satya *et al*., 2014).**

$$\text{inhibition \%} = \frac{C - T}{C} \times 100$$

Where C is the average of three replicates of hyphal extension (mm) of control and T is the average of three replicates of hyphal extension (mm) of plates treated with tested material (total flavonoids extract of *Carthamus tinctorius* L.). EC_{50} values were determined by the linear regression (LPD line computer program) of the probit of the tested fungus percentage inhibition vs. Logs the concentrations (ppm) of the prepared total flavonoids extract . The EC_{50} notation used to indicate the effective concentrations (ppm) that causes 50% growth inhibition. In essence, the lower value of EC_{50} is the highest efficacy of prepared total flavonoids extract in the test under consideration.

2.5. Antioxidant activity

2.5.1. The 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

The antioxidant activity of the total flavonoids extract from safflower flower was assessed by their ability to scavenging 2.2-diphenyl-2-picrylhydrazyl hydrate (DPPH) stable radicals as reported earlier (**Mimica-Dukic *et al*., 2003**). Total flavonoids extract (250µg/ml) was mixed with DPPH solution (1ml; 90µM) and then with methanol 95% to a final volume of 4ml. Synthetic antioxidant butylated hydroxyl toluene (BHT) was used as a positive control. After 1h incubation period at room temperature, the absorbance was recorded at 515nm. Percent radical scavenging concentration was calculated using the following formula:-

$$\text{radical scavenging \%} = \frac{\text{A (blank)} - \text{A(sample)}}{\text{A(blank)}} \text{X100}$$

A (blank) = Absorbance of the control
A (sample) = Absorbance of the test sample

2.6. Identification of total flavonoids extract by HPLC

Flavonoids compounds of *Carthamus tinctorius* L. was identified by high-performance liquid chromatography (HPLC) analysis in Food Technology Res. Institute, Agriculture Res. Center according to the method described by **Plazonic *et al*., (2009).** HPLC apparatus used Hewlett Packard series 1050 equipped with auto sampling injector, solvent degasser, ultraviolet UV detector set at 330nm and quarter HP pump series 1050. The column temperature was maintained at 35°C. Gradient separation was carried out methanol and acetonitrile as a mobile phase at flow rate of 1ml/min. Flavonoids standards from Sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate flavonoids compounds by the data analysis of Hewlett Packard software.

3.Results

3.1. The yield of flavonoids

The percentage yield of total flavonoids (precipitate from 70% ethanol extract of *Carthamus tinctorius* L. using lead acetate method) was 14%, as recorded in Table (1).While, **Sabah and Saleh,(2015)** obtained that the flavonoids isolated from the dried petals of safflower was recorded 20.3%. The differences in composition of certain plants might be attributed to the environmental variations, extraction process, nature of the extraction solvent , age of the plant, and part of the plant used. This observed in according with **Womeni *et al*., (2013)**.

Table(1). The percentage yield of total flavonoids from *Carthamus tinctorius* L. flowers

extract	Wt. of plant (g)	Wt. of extract (g)	Percentage yield (%)	Color of matter
Flavonoids	25.0	3.5	14	yellow

3.2. Antioxidant activity

DPPH is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants comparing with a positive control BHT. The experimental data of total flavonoids extract from *Carthamus tinctorius* L. flowers revealed that total flavonoids showed 88.1% antioxidant activity while, BHT showed 89.5% antioxidant activity, as shown in Table (2). This result, illustrated that the total flavonoids of *Carthamus tinctorius* L. has a strong effect of scavenging free radical. This result in an agreement with **Sabah and saleh,** (**2015**).

Table(2) Antioxidant activity of total flavonoids from *Carthamus tinctorius* L.

Tested sample	DPPH radical scavenging (%)
Total flavonoids	88.1
BHT	89.5

Various studies suggested that total phenolic compounds are closely associated with antioxidant activity. Phenols take part in biological oxidation-reduction reactions following the quinine/hydroquinone mechanism (**Duh and Yed, 1997**). Antioxidant and antifungal activities of the plant extracts are great interest in both fundamental science and food industry, since their use as potential natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones (**Maskovic *et al*., 2011**).The antioxidant activity of flavonoids might exert modulatory activities in cells through cell signaling acting mainly in protein kinase. These interactions could trigger unpredictable results, depending on cell type cell cycle and the kind of stimulus applied **(Kyselova, 2011)**.The water soluble antioxidants and free radical scavengers have potent role in prevent oxidative cell damage **(Okwe and Okue, 2004).**

3.3. Antifungal activity

Antifungal activity of total flavonoids extract from *C. tinctorius L.* was recorded. In the present study, total flavonoids extract at five concentrations (100,200,300,400 and 500µg/ml) were tested against eight selected phytopathogen fungi, as shown in Table **(**3**)**

The results in Table **(**3**)**. Indicated that total flavonoids extract from *C. tinctorius L*. showed antifungal effects against all the tested phytopathogenic fungi comparing with control. EC_{50} values ranged from 235.2 to 485.1ppm Inhibition activity of selected fungi enhanced with an increase concentrations of total flavonoids extract. *Alternaria alternate, Sclerotium rolfsii, Sclerotonia sclerotorium* and *Fusarium oxysporium* were most sensitive fungi to total flavonoids extract comparing with the other tested fungi which EC_{50} were 235.2, 280.7, 281.9 and 321.3ppm, respectively. On the other hand, *Rhizoctonia solani* was more tolerance to total flavonoids extract which EC_{50} was highest concentration (485.1ppm) comparing with the other fungi.

According to reference data, several studies on pharmacological activities from flowers of *Carthamus tinctorius* L. indicated that it has several activities in both *in vitro* and *in vivo* tests, for example, antifungal and antioxidants **(Sabah and saleh, 2015)**. Antifungal activity of the flavonoids extract was enhanced by increasing the concentrations of the extract. The activity was attributed to the presence of phenolic compounds which can use as a natural fungicidal agent against common pathogens of crops **(Fardos, 2009)**. The mechanism of actions may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical and often leading to the inactivation of the protein and loss function. They have the ability to form complexes with extracellular and soluble proteins and to make complex with microbial membranes **(Ali, 1999).**

3.4. Identification of flavonoids compounds by HPLC.

HPLC analysis of total flavonoids extract from *Carthamus tinctorius* L. in Table (4) revealed that safflower flower contains 17 flavonoids compounds. Hespirdin is the major compound which (8813.28ppm) followed by quercetrin (1000.50ppm), Apig-6-arbinose-8-glactose (631.49ppm) and rutin (513.06ppm). On the other hand, safflower flower contains low amounts of

Table (3): Antifungal activity of total flavonoids extract from (*Carthamus tinctorius* L.) on some selected phytopathogenic fungi.

Fungi	%Inhibition growth at different concentrations(µg/ml)					EC_{50}
	100	200	300	400	500	
Sclerotium rolfsii	22.2	40.0	54.4	72.2	83.3	280.7
Rhizoctonia solani	10.0	20.0	26.7	42.2	51.9	485.1
Sclerotonia sclerotorium	27.8	38.9	56.4	72.2	88.9	281.9
Fusarium solani	11.1	20.0	44.4	61.1	77.0	350.6
Fusarium oxysporium	16.6	41.1	58.9	70.2	81.6	321.3
Humicola fuscoatra	14.4	30.0	38.9	55.6	72.2	370.8
Alternaria alternata	26.7	44.4	63.3	83.3	94.4	235.2
Botrytis cinerea	11.1	21.1	37.8	51.1	61.2	384.7

quercetin, apegnin, kaempferol and naringenin which recorded 15.01, 15.74, 17.22 and 26.40ppm, respectively.

Table 4. HPLC analysis of total flavonoids extract from *Carthamus tinctorius* L.

Flavonoids	Concentration (ppm)
Apig.6-arbinose 8-glactoside	631.49
Apig. 6-rhamnose 8-glucoside	301.74
Naringin	497.79
Hespirdin	8813.28
Rutin	513.06
Quercetin-3-O-glucoside	64.72
Apig.7-O-neohespiroside	188.73
Kamp.3.7-dirhamoside	49.17
Quercetrin	1000.50
Apigenin-7-glucoside	59.85
Acacetin 7neo hesperside	37.76
Kaemp.3-2-p-comaroyl glucoside	361.10
Quercetin	15.01
Naringenin	26.40
Hespirtin	50.90
Kaempferol	17.22
Apegnin	15.74

From literature data, Carthamin, a flavonoid type of dye is the main constituents in *C. tinctorius* flowers (**Shirwaikar *et al*., 2010).** Also, The flowers contains cathamidin, isocarthamidin, quercetin, kaempferol and 6-hydroxy kaempferol (**Jin *et al*., 2008**). The flavones luteolin and its glucopyranoside (i.e. luteolin-7-o-beta-D-glucopy-ranoside and luteolin-7-o-(6-o-acetyl)–beta-D-glucopyranoside) were found in *C. tinctorius* flowers (**Xiao and Liu, 2005**). The flowers of this plant contains acacetin, luteolin, rutoside, nicotiflorin, cynaroside and quercetin (**Yoon *et al.,* 2007 *and* Yue *et al.,* 2013**). Additionally, **Salem *et al*.,** (**2014**) showed that safflower flower contains epicatechin, resorcinol, quercetin-3-galactoside, quercetin-3-rhamnoside, luteolin and quercetin.

Based on the results of the present study, it can be concluded that the total flavonoids extract from *Carthamus tinctorius* L. flowers possesses have strong antioxidant and antifungal activities. These effects may be attributed to the presence of glycoside compounds such as hespirdin and quercetrin, which can use as a natural fungicidal agent against common pathogens of crops. So that, pure compounds should be submitted to *in vitro* and *in vivo* assays to draw conclusions on the molecular mechanisms of action of flavonoids.

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التركيب الكيماوي والنشاط البيولوجي للفلافونيدات المستخلصة من أزهار نبات العصفر

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قسم بحوث المبيدات الفطرية والبكتيرية والنيماتودية

المعمل المركزي للمبيدات – مركز البحوث الزراعية

الدقي – الجيزة – مصر

الملخص العربي

تم تقييم الفلافونيدات المستخلصة من أزهار نبات العصفر من حيث فعاليته كمواد مضادة للاكسدة ومقاومة للفطريات الممرضة للنبات. أظهرت نتائج البحث أن الفلافونيدات المستخلصة تمثل 14% من نبات العصفر. أما فعالية الفلافونيدات تمثل 88.1% كمواد مضادة للأكسدة . وكذلك تم اختبار فعالية الفلافونيدات المستخلصة بالتركيزات 100، 200، 300، 400، 500 ميكروجرام /ملي كمواد مضادة للفطريات الممرضة للنبات .أثبتت النتائج أن الفطريات *Alternaria alternate, Sclerotium rolfsii, Sclerotonia sclerotorium* and *Fusarium oxysporium* من اكثر الفطريات حساسية للفلافونيدات المستخلصة مقارنة بالفطريات الأخري المختبرة وكانت التركيزات 235.2، 280.7 ، 281.9 ، 321.3 جزء في المليون علي التوالي. بينما فطر *Rhizoctonia solani* من اكثر الفطريات مقاومة الفلافونيدات حيث كان التركيز 485.1 جزء في المليون . بالأضافة الي ذلك تم التعرف علي مركبات الفلافونيدات المستخلصة باستخدام HPLC analysis ووجد أن أزهار نبات العصفر يحتوي علي 17 مركب فلافونيد ، وتعتبر Hespirdin هو المركب الرئيسي حيث قدرت نسبتة(8813.28 جزء في المليون) ويلية مركب quercetrin) 1000.50 جزء في المليون(، مركب Apig-6-arbinose-8-glactose)631.49 جزء في المليون(ومركب rutin)513.06 جزء في المليون(.ونستنتج من هذة الدراسة ان الفلافونيدات المستخلصة من أزهار نبات العصفر لها فعالية كمواد مضادة للاكسدة ومقاومة للفطريات الممرضة للنبات وقد ترجع هذه الفعالية الي المركبات الجليكوزيدية .