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Estimated Potential Antioxidant activity of *Cuscuta chinesis* Lam. Methanol – Watery Extract

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بحث مقدم

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وما أوتيتم من العلم إلا قليلاً

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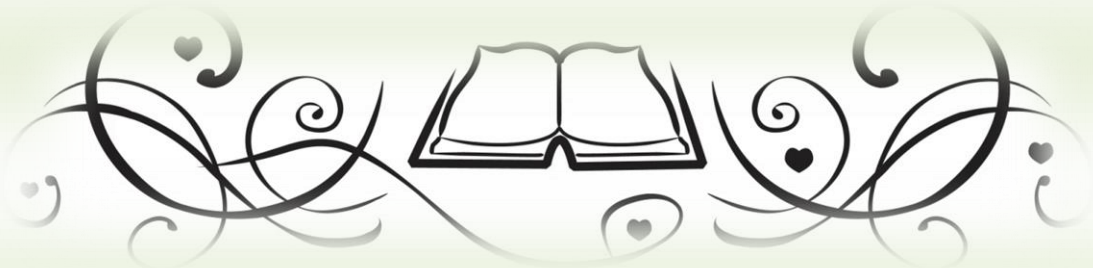

اهداء

إلى من يسعد قلبي بلقياها

إلى روضة الحب التي تنبت أزكى الأزهار

أمي

إلى رمز الرجولة والتضحية

إلى من دفعني إلى العلم وبه ازداد افتخار

أبي

الشكر والتقدير

الحمد لله رب العالمين ، والصلاة والسلام على رسوله محمد

(صلى الله عليه وسلم)

الشكر الجزيل والتقدير ا للدكتورة فادية حميد محمد ،

شكرا جزيلا لجميع أساتذتنا في كلية العلوم ، قسم علم الحياة وكل

زملائنا كانوا مخلصين في تقديم الدعم أو المشورة للتغلب

... على الصعوبات في جميع مراحل الدراسة

The Content

Estimated Potential Antioxidant activity of *Cuscuta chinesis* Lam. Methanol – Watery Extract

Abstract

The Potential Antioxidant activity of *Cuscuta chinesis* Lam. Methanol – Watery Extract was estimated by using DPPH assy. The percentage of DPPH scavenging activity was investigated for Serial concentrations ranged of extract (3.125, 6.25, 12.5, 25, 50, 100 and 200) µg/ml . IC50 in µg/mL was calculated as an amount of antioxidant present in the sample necessary to decrease 50% the initial DPPH concentration .The results revealed that methanol-watery extract of *C. chinesis* had a strong anti-oxidant activity by showing the high percentage of DPPH scavenging activity reached to (**82%**) in the concentration 200 µg/ml and The IC50 value was **39.226** µg/ml and by results we conclude the extract had a strong antioxidant activity.

Introduction

Oxidative stress (OS) plays a major role in weaken and destruction all cells and organs of human, therefore injury and development of degenerative ailments and chronic diseases such as arthritis, aging, autoimmune disorders, neurodegenerative , cardiovascular disorders and cancer (Khalid,2007). Although cells are equipped with an impressive antioxidant enzymes as well as non enzymetic small antioxidant molecules, these agents may not be enough to normalize the redox status under oxidative stress caused under adverse physicochemical, environmental or pathological conditions, when either the generation of free radicals is enhanced or their scavenging is inhibited. Under these circumstances

supplementation with exogenous antioxidants is required to restore the redox homeostasis in cells (Patel *et al* ., 2010).

Many medicinal plants investigated in search of novel antioxidants. Plants synthesize several antioxidants to them against damage caused by active, reactive oxygen species (ROS) (Rad and Sen ,2013). These compounds include chlorophyll derivatives, alkaloids, essential oils, phytosterols , Phenolics, terpenoids and polyphenolics(Sharifi *et al*., 2014; Rad ,2013). Some of antioxidants phytochemicals isolated from plants include curcumin, eugenol, flavonoids, coumarins, carotenoids, tannins, gallic acid, limonene, terpenoids, β- sitosterol etc (Gupta and Sharma ,2006). Use of plants as a source of medicine has been an ancient practice

and is an important component of the health care system in India (Sharifi *et al*., 2014). Various plant extracts and oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Rad *et al*.,2014).

Cuscuta chinensis Lam. or Dodder plant, is an annual voluble parasitic plant of the family Convolvulaceae, its leafless plant and its unable for metabolism , so it uptake nutrient that ready to absorption from his host plant by wrapping his parasitic stem around host plant and sucking nutrients by haustorians (Mavlonov *et al*.,2008) . *Cuscuta chinensis* Lam. has found its use as a traditional medicine in China, Korea, Pakistan, Vietnam, India and Thailand. It was commonly used as an anti-aging agent, anti-Inflammatory agent and pain reliever (He *et al*., 2010).

Around 2000 years ago, *Cuscuta chinensis* seeds was recorded in Shen Nong's Herbal, the ancient Chinese herbal classic for the first time in

Chinese history. It was listed as a drug for the kidney-tonifying and liver-strengthen functions (Mou, 2002). *C. chinensis* has an ability to improve vision, stop diarrhea and prevent abortion. (Teng, 2007). In the famous classic book of Chinese Materia Medica referred to the effect of *C. chinensis* to improve eye sight and prevent aging, tonify the muscles, enhance the activity of bone and tendon ,principally and used to treat or improve excessive cold in male and female reproductive organs ,spermatorrhea ,frequent urination, thirst, mouth's bitter taste feeling or aggregation of the blood due to cold (Li,2009).Although presence of many therapeutic effect of *Cuscuta chinensis* seeds , alitle study was done about whole *Cuscuta chinensis* antioxidant and therapeutic effect ,so our study aimed to detect about Potential antioxidant of methanol – watery extract of *C. chinesis* whole Plant and determined if was a strong scavenger Antioxidant by estimate antioxidant IC_{50} value by using DPPH* scavenging activity assay

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DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1 picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical molecules. It used as a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay (Sharma and Bhat ,2009) .

Materials and methods

Plant Collection , identification and drying

The whole plant of *Cuscuta chinesis* Lam. was collected at duration mid of November to mid of December 2022 from gardens of Babylon university , then the plant was identified by Dr. Nedaa Adnan (Plant herbarium / department of biology / college of science / university of Babylon)The collected plant was dried in shad at room temperature for 10 days . Dried plant was milled by using electric miller.

Plant extract preparation

The dried powder of *Cuscuta chinesis* Lam. was extracted with solvent methanol – water (1:1 v/v) according to Ekpenyong *et al* (2012) . 1g of plant powder : 10 ml of solvent was blended for 30 min at room temperature. The suspension was filtered by guise and the filtrated liquid was concentrated to dryness in oven at 45 C° . the dried concentrated material was milled by using electric miller and the final powder was sterilized by UV equipment for 20 min (Ekpenyong *et al* ., 2012).

DPPH Radical Scavenging activity

The free radical scavenging of *C. chinesis* Methanol – Watery Extract was measured by 1-diphenyl -2-picrylhydrazl (DPPH) scavenging activity assay. One milliliter of 0.1 mM solution of DPPH in methanol was added to 2 ml of *C. chinesis* Methanol – Watery Extract with the following concentrations (200 ,150 ,100 ,50 ,25 ,12.5 ,6.25 ,3.125 µg/ml); after 30 min, absorbance was measured at 517 nm.

All concentrations of the extract were tested three times. Percentage reduction of DPPH (Q) was calculated according to the formula below (Oktay *et al*., 2003).

Q =100 × (A0 - AC) / A0

Where: A0= Absorbance of control

AC=Absorbance of the two samples after 30 min incubation.

IC50 values denote the concentration of sample required to scavenge 50% of DPPH free radicals. IC50 value was determined from the plotted graph of scavenging activity against the different concentrations, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The measurements were triplicates and their scavenging effect was calculated based on the percentage of DPPH scavenged (Singh *et al*., 2008; Priyanka *et al*., 2011).

Results

DPPH scavenging activity assay

The dose response curve of methanol - watery extract of *C. chinesis* was measured using 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity test, results are expressed as mean Table (3-1).

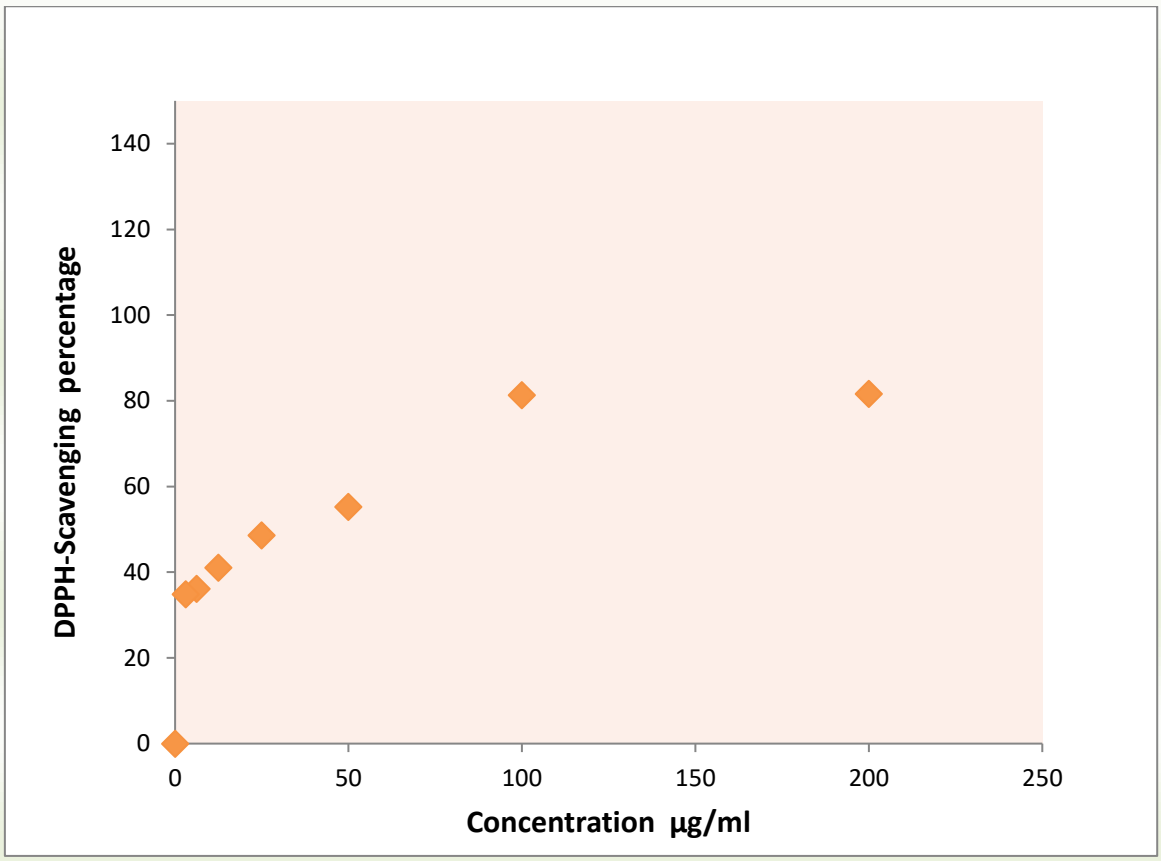
The percentage of DPPH scavenging activity was investigated for Serial concentrations ranged of extract (3.125, 6.5, 12.5, 25, 50, 100 and 200) µg/ml and scavenging percentage was (35, 36, 41, 49, 55, 81, 82)% respectively Table (3-1).

IC50 in µg/mL as amount of antioxidant present in the sample necessary to decrease 50% the initial DPPH concentration was calculated by the linear regression equations, (Figure3-1) Where:

Y= Percentage of DPPH scavenging activity

X=concentration.

By applicate the liner regression equation of each tested agent by considering Y is to be 50%, We got IC50 of *C. chinesis* methanol - watery extract which was **39.226** µg/ml.



Figer (3-1) Response curve of different concentration of *Cuscuta chinesis* Methanol – Watery Extract on DPPH free radical scavenging activity

Table (3-1) The percentage of DPPH scavenging activity for different concentration of *Cuscuta chinesis* Methanol – Watery Extract

Concentration (µg/ml)	Mean of AB under 517 nm	Scavenging percentage
0	0.633	0 %
200	0.116	82 %
100	0.118	81 %
50	0.283	55 %
25	0.325	49 %
12.5	0.373	41 %
6.25	0.404	36 %
3.125	0.412	35 %

Discussion

DPPH, a highly stable free radical has been widely used to assess the antioxidant potential of many natural products (Maurya and Rizvi ,2009). The effect of antioxidants is considered to be due to their hydrogen donating ability to the DPPH free radical. IC50 in µg/mL was calculated for methanol-watery extract of *C. chinesis* as amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration .The results revealed that methanol-watery extract of *C. chinesis* had a strong anti-oxidant activity by showing the high percentage of DPPH scavenging activity reached to(82%) in the concentration 200 µg/ml as shown in table (3-1).and from figure (3-1) and by the liner regression equation of each tested agent by considering Y is to be 50%. The IC50 of DPPH for *C. chinesis* methanol - watery extract was **39.226** µg/ml. the strong antioxidant activity of *C. chinesis* methanol - watery extract may be regard to the presence of berberine (strong antioxidant alkaloid phytochemical) in a high concentration in awhole *C. chinesis* plant as improved in **(**Alsultany , 2018**)** and others like Sineeporn *et al* in (2014) and Kwon *et al* (2000) who confirm the presence of berberine in *C. chinesis* Lam. plant when they screening about many phytochemical compound in *C. chinesis* Lam. plant and they got that *C. chinesis* contain many important phytochemical compound one of them was the berberine .

Quercetin is a polyphenolic phytochemical. It had chemical structure that stops oxidation by acting as a scavenger of free radicals which responsible for oxidative chain reactions, so it regard as astrong antioxidant compound (Prabhu *et al.*, 2017)the presence of Quercetin in *C. chinesis* was confirm by many researchers like (Ye *et al.*, 2002 and Sineeporn *et al* ,2014) and they

regarded it as a one of a causative effect of anti-oxidant activity of *C. chinesis* plant. Other cause for this a strong antioxidant activity of *C. chinesis* plant may be regard to beta-carotene (Alsultany, 2018). Many nutrition scientist regard the yellow/orange color of fruits, vegetables and herbs indicate to presence of high amount of beta-carotene (Yen *et al.*, 2008). The yellow/orange color of *C. chinesis* plant was regarded as a confirmed indication about the presence of high amount of beta-carotene and this suggest was Supported by the study of (Schierle *et al*., 2004) who showed that *C. chinesis* plant was a good source of pro-vitamin A(β – carotene).

C. chinesis has ability to plucked off the most host efficacious compound, making the interesting that the parasite's medicinal benefits are determined by its host (Lin *et al* ., 2007).and recent study by El-Sayed *et al* (2012) concluded that different parts; leaves, stems, fruits and flowers of *Conocarpus erectus* L. (the host plant); have phenolic compounds especially tannins , alkaloids, Coumarins and Terpenes and he referred to the antioxidant, anticancer and antimicrobial properties of this plant.

Support study submitted by Donnapee *et al* (2014) confirm that *C. chinesis* contain many phytochemical compounds like flavonoids ,phenolic acids ,steroids ,hydroquinones , volatile oils, lignans ,alkaloids ,polysaccharides, resin, glycosides and fatty acid which giving antioxidant and Pharmacological Activities of its extracts. All that justified the strong antioxidant activity of *C. chinesis* plant or its extract and the possibility of it to reduce oxidative stress.

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