

Determination of total phenol, antioxidant and antimicrobial activities of *Avena sativa* and *Ocimum basilicum*

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Abstract:

The present study investigated the total content of phenolic compounds, antioxidant and antimicrobial activities of water extracts oat (*Avena sativa*) and basil (*Ocimum basilicum*), medicinal plants. The Folin-ciocalteu reagent assay was used to estimate the total phenolic content of plants extract. The antioxidant capacity of the plants extract was tested by ferric reducing/antioxidant power Assay (FRAP) and ferric reducing scavenging activity using DPPH method, and the antimicrobial activity was measured against [*Staphylococcus epidermidis*; *Staphylococcus aureus*; *Proteus spp.*; *Klebsiella spp.*; *Escherichia coli*; *Candida albicans*] as tester strains. The total phenolic content of *Avena sativa* and *Ocimum basilicum* extracts revealed that the mixture of plants showed higher content. The mixture of *Avena sativa* and *Ocimum basilicum* extracts showed the highest antioxidant capacity followed by *Avena sativa* extract and *Ocimum basilicum* extract with FRAP and DPPH assay. However, mixture of *Avena sativa* and *Ocimum basilicum* extracts exhibited the highest antimicrobial activity when compared to the other extracts. Thus the study revealed that the consumption of mixture herbs may enhance the immune power of our body against diseases due to free radicals.

Key words: Antimicrobial, antioxidant, total phenolic content, oat, and basil.

Introduction:

Nowadays, increasing utilization of synthetically chemical leads to several diseases and high morality of mankind such as cancer, heart diseases, and malfunction of immune system. Plants produce secondary metabolites to protect themselves. These products are known by their activity substances such as phenolics, alkaloids, and terpenoids [1]. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens including bacteria, fungi, and virus [2,3]. However, the antimicrobial activity of several extracts of different plants was reported such as the crude water extract of garlic and clove possesses antimicrobial activity [2].

The secondary metabolites of plants can be antioxidants, which scavenge free radicals, and toxin for other organisms. The body has a defense system of antioxidants to get rid of reactive free radicals, if they were not excess [4]. The antioxidant activity of plant phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals. It will be beneficial to human.

The oat (*Avena sativa* L.) and basil (*Ocimum basilicum* L.) plants have long been part of Iraqi cultures as they were consumed regularly as part of the diet. These plants were eaten raw as salad, or used in cooking to flavor the dishes. Some of these plants have been used as folk remedies for the treatment

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of ailments such as diabetes, high blood pressure, arthritis and fever as well as health tonic [5]. It was now known that these plants contain a wide variety of biologically active photochemical constituents.

This study aimed to estimate phenolic compounds, antioxidant capacity and antimicrobial activity of water extracts from mixture of oat and basil compared to the other extracts.

Materials and Methods:

Samples of oat and basil were purchased from a local market in Baghdad city, Iraq. Plants were air dried at room temperature and grinded into powder by using coffee grinder. All of applied chemicals were of pro analysis purity and were purchased from Fluka chemie (buchs Switzerland).

Preparation of methanol extract

The powdered plant material (25mg) was extracted with 250 ml of 70% methanol at room temperature for 24 hours. After filtering and rotary evaporator to dryness, the crude extracts were obtained [6].

Determination of total phenolic content

The total phenol was determined using the Folin-Ciocalteu method. 0.5ml of plant extracts was taken in test tubes and 5ml of Folin-Ciocalteu reagent and 4ml of aqueous sodium carbonate were added to the tubes. The tubes were kept at density was measured 745 nm using spectrophotometer [7].

Total phenolic contents were determined as a gallic acid equivalent (GAE) based on Folin-Ciocalteu calibration curve using gallic acid (ranging from 0 to 250 mg/l) as the standard and expressed as mg gallic acid per gram of dry sample [8].

Determination of radical scavenging activity (DPPH assay)

The free radical-scavenging activity of the *A. sativa* and basil *O. basilicum*

methanol extract was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [9]. One-tenth mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1 to 5 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = (1 - A_{\text{sample}} / A_{\text{control}}) \times 100$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Determination of Ferric Reducing Antioxidant Power (FRAP assay)

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain [10] as a measure of (antioxidant power). The FRAP assay measures the change in absorbance at 593nm owing to the formation of a blue colored Fe^{II} -tripyridyltriazine compound from the colorless oxidized Fe^{III} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100–1000 $\mu\text{mol/L}$) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the antioxidant tested was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration, this representing a one-

electron exchange reaction. The results were corrected for dilution and expressed in $\mu\text{mol Fe}^{\text{II}}/\text{L}$. The sample to be analyzed was first adequately diluted to fit within the linearity range.

Antimicrobial assay

The antimicrobial assay was performed via agar-well diffusion method against some of pathogenic bacteria and fungi. One loopful of microbial was pre-cultured in Muller Hinton broth for 24 h and turbidity of culture was adjusted to optical density of McFarland 1.5×10^8 cfu/ml of pathogenic bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus spp.*, and *Klebsiella spp.*) and 1.5×10^3 cfu/ml of pathogenic yeast (*Candida albicans*). Then, 0.1 ml of pre-cultured bacterial suspension was seeded on Muller Hinton agar plates. The dried extracts were impregnated on the surface of the incubated agar plate by a dispenser, then incubated for 24 hrs at 37°C. The inhibitory zone around the halo was measured. commercial antibiotics, streptomycin (Merck, Germany) were used as a positive control [11].

Statistical analysis

Analysis of variance (ANOVA) was performed to test whether group variance was significant or not, according to Al-Mohammed *et al.*, [12].

Results and discussion:

Total phenolic content (TPC) of the extracts were determined by Folin-Ciocalteu method and expressed in mg GAE/100 g. Total phenolic content is higher in mixture of *A.sativa* and *O. basilicum* extract (200 ± 0.09) as compared to *A.sativa* (195.12 ± 0.7) and *O. basilicum* (122.34 ± 0.3). Antioxidant activities of *A.sativa* and *O. basilicum* extracts were evaluated by DPPH and FRAP assays, and the results are shown in Table 1. In DPPH assay, the mixture of *A.sativa* and *O. basilicum* showed highest activity (95.6 ± 2.4) followed by *A.sativa* (91 ± 0.2) and *O. basilicum* (88.4 ± 0.7). Whereas in FRAP assay, mixture of *A.sativa* and *O. basilicum* exhibited highest activity (77.5 ± 0.9), followed by *A.sativa* (66.3 ± 1.2) and *O. basilicum* (46.2 ± 0.4).

Table (1): Total phenolic content(TPC), radical scavenging activity (DPPH inhibition), and Ferric Reducing Antioxidant Power (FRAP) of *Avena sativa* and *Ocimum basilicum* extracts.

Species	TPC(mg GAE/100 g)	Antioxidant activity	
		DPPH inhibition(%)	FRAP(mmol/L)
<i>Avena sativa</i>	195.12 ± 0.7	91 ± 0.2	46.2 ± 0.4
<i>Ocimum basilicum</i>	122.34 ± 0.3	88.4 ± 0.7	66.3 ± 1.2
Mixture of <i>Avena sativa</i> and <i>Ocimum basilicum</i>	200 ± 0.09	95.6 ± 2.4	77.5 ± 0.9

The results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. Phenolic compounds, such as flavonoids, phenolic acid and tannins possess diverse biological activities, such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic activities. These activities might be related to their antioxidant activity [13]. Phenols are very important plant constituents

because of their scavenging ability owing to their hydroxyl groups. Phenolic compounds from plants are known to be good natural anti-oxidants [14]. Our findings agree with the widely accepted idea that antioxidant activities of botanical extracts are contributed by polyphenols [15].

Plant extracts showed varying degrees of antimicrobial activity on the microorganisms tested as shown in table 2.

Table (2): Antimicrobial activities of methanol extracts of *Avena sativa* and *Ocimum basilicum* against 6 pathogenic organism (inhibition zone diameter in mm)

Test microorganisms		Extract concentration 10mg/ ml			positive control 10mg/ml
		Avena sativa	ocimum basilicum	Avena sativa + ocimum basilicum	Streptomycin
Gram -	<i>Staphylococcus epidermidis</i>	18.3	20.1	22	20
	<i>Staphylococcus aureus</i>	16.4	15.5	20.3	23
Gram +	<i>Proteus sp.</i>	14	12.6	14.5	20
	<i>Klebsiella sp.</i>	16.4	15.3	19.6	20
	<i>Escherichia coli</i>	18.5	18.1	18.3	22
F	<i>Candida albicans</i>	15.6	13.7	18.4	31

Generally, the mixture of *A.sativa* and *O. basilicum* methanol extract was more effective than the single *sativa* and *O. basilicum* extracts on the growth of pathogens. The less effect of the single *sativa* and *O. basilicum* extracts may be due to the quality and quantity of the active compounds extracted by water. While mixture of extract showed an inhibitory action against gram positive bacterium activity as well as against gram negative bacterium. It also showed pronounced activity against the fungus *C. albican*, thus demonstrating a broad spectrum activity against organisms. This may be attributed to the presence of Phenols and phenolic compounds which are known to have antibacterial and antifungal properties [16].

Table (2) appears that mixture of *A.sativa* and *O. basilicum* extracts are more efficient than other extracts; the reason for this may be due to the compounds already extracted by methanol particularly phenolic compounds. The site and number of hydroxyl groups on the phenol are thought to relate to their relative toxicity to microorganisms, with that increased hydroxylation results in increased toxicity. Jawety *et al.*, [17] described the mechanism thought to be responsible for phenolic toxicity against microorganisms to membrane disruption, binding or adhesion making complex with cell wall, inactivation of enzymes, and binding to proteins.

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تحديد مجموع الفينولات ومضادات الأكسدة ومضاد بكتيري لنبات الشوفان والريحان

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الخلاصة:

أظهرت الدراسات الحالية وجود مجموع مركبات فينول، وفعالية كمضادات للأكسدة و ميكروبية في نباتات الطبية لمستخلص المائي لشوفان والريحان. استخدم كاشف فولنك لتحديد محتويات الفينول لمستخلص النباتات. وفعالية لإزاحة الجذور الحرة FRAP واختبرت كفاءة مستخلص النباتات كمضاد للأكسدة بواسطة تحليل *[Staphylococcus epidermidis]*، وفعاليته كمضاد ميكروبي ضد سلالات DPPH باستخدام طريقة *Staphylococcus aureus; Proteus spp.; Klebsiella spp.; Escherichia coli; Candida albicans*. وجد أن مجموعة محتويات الفينول لخليط مستخلصي الشوفان والريحان تحتوي على أعلى تركيز. ووجد أن خليط مستخلصي الشوفان والريحان أكثر فعالية كمضاد للأكسدة ويليهما مستخلص شوفان والريحان. وكذلك بينت الدراسة إن مستخلص خليط أكثر فعالية كمضاد ميكروبي عندما DPPH و FRAP بفحص ال مقارنة بمستخلصات الأخرى. أوضحت هذه الدراسة أن استهلاك خليط من النباتات الطبية ربما يساعد في تقوية مناعة لجسم ضد الأمراض التي تسببها جذور الحرة.