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EVALUATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOIDS CONTENT AND FREE RADICAL SCAVENGING ACTIVITY OF LEPIDIUM SATIVUM L. SEEDS AND LEAVES PLANTED IN SYRIA

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Most of previous research has focused on the seeds of Lepidium sativum L., whereas few ones were interested in the chemical content of its leaves. This research study determines total phenolic content, total flavonoids content in the methanolic and chloroformic extracts for the seeds and leaves of Lepidium sativum. Antioxidant activity of the previous extracts has also been evaluated by determining free radicals (DPPPH assay) scavenging activity. The results have shown that the methanolic and chloroformic extracts from seeds has phenolic content about (1043± 0.16mg GAE\100g) and (306.12± 0.12mg GAE\100g) respectively. Whereas, lower quantities were found in leaves (993.40± 0.16 mg GAE\100g) and (85.86± 0.01mg GAE\100g) for methanolic and chloroformic extracts respectively. However, the flavonoids content in leaves (153.32± 0.60 mg QE\100g, 70.94± 0.71mg QE\100g) was higher than in seeds (28.76 ± 0.01 mg QE\1oog ,8.38± 0.12mg QE\100g). Also, the free radicals scavenging was found to be (IC50=126.44±0.63µg\ml methanol) and (IC50=50.97±0.14µg\ml methanol) for leaves and seeds respectively. According to the results above, Lepidium Sativum can be considered as natural antioxidant and important nutritional supplements.

INTRODUCTION

Plants remain one of main source for medical and pharmaceutical compounds[1]. Nowadays, most of cancer and antimicrobial drugs are reported to be either natural products or derived from them, as it is believed that the usage of medicinal plant for health care has less side effects[1&2]. The Brassicaceae family is known for its nutritional benefits and therapeutic effects such as antimicrobial, antiviral and anti-inflammatory[3&4].

Brassicaceae is a large angiosperm dicot family of plant kingdom[4]. It includes 372 genera[5]; one of the largest genus in the Brassicaceae family is *Lepidium* which include 234 species distributed mostly in temperate and subtropical regions[4&6]. *Lepidium sativum* is one of the most common species in this genus, which is considered one of the medicinal plants in traditional medicine[7&8].

Lepidium sativum L. (Garden cress) is annual herb with a height of 50-80 cm[9]. It is erect gray green plant[7&9], stem is cylindrical branched and glabrous[8]. Its leaves are pinnate small and bright green which are arranged alternately or opposite to each other[8]. The flowers are bisexual with white petals and the inflorescence is in recemes[7]. The fruits are circularity flattened and pale green siliquaes, each siliquae has two seeds[9]. Seeds are small, oval-shaped, smooth and reddish brown[9].

The origin of *Lepidium sativum* is thought to be Ethiopia or southwest Asia and then spread to western Europe and various part of the world[7&9]. It can be cultivated around the whole year. However, it prefers cultivation at autumn (November) or winter (February) to get the best harvest[7&9].

Lepidium sativum has been mentioned by many civilization. The ancient Egyptians and

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Persians used Cress (*Lepidium sativum*) as food source, also Islamic scientists used it to manage stomach ache. The Mediterranean's used this plant to protect crop from insects and pests[9]. While, the Indians considered that *Lepidium sativum* as a kind of spices for its pungent test[8]. *Lepidium sativum* have important utilizations in traditional medicine, such as local application to relief of inflammatory and rheumatic pains. Moreover, the seeds are used as laxative, carminative, galactagogue, aphrodisiac and tonic[8]. They are also useful as poultices for curing the skin diseases[10]. Many recent research showed that *Lepidium sativum* seeds have anti-inflammatory properties, it reduces inflammatory and rheumatic pain in rheumatoid arthritis[7&9&10]. Also it has antimicrobial activity against (*Escherichia coli*, *Pseudomonas aeruginosa*, *Satphyloccus aureus*, *Klebsiella pneumomea*)[7]. The seeds can be used for management asthma attacks because their bronchodilator effect[7]. Moreover, Cress has diuretic, anti hypertensive, hepato -protective, hypoglycemic, anticancer and antioxidant effects[7-9]. These pharmaceutical effects are due to phytochemical constituents of *Lepidium sativum* which contains Glucosinolates, the major compounds in plants of Brassicaceae family. Gluconasturin and glucotropaeolin were isolated from the seeds, while ethyl butyl glucosinolate, methyl glucosinolate and butyl glucosinolate were isolated from the aerial parts[11]. Maier et al observed that the seeds contain imidazole alkaloids (Lipidine A, B, C, D, E, F)[12]. Moreover, the plant contains phenolic compounds represented by phenolic acids (sinapic acid and its choline ester Sinapin, ferulic acid) coumarins and flavonoids (5,4-dihydroxy-7,8,3,5tetramethoxyflavones, Kaempferol glycozides)[7&10&13].

Although, *Lepidium sativum* is cultivated and distributed to various nearby countries and most agricultural areas in Syria. It is characterized by nutritional importance and popular medical utilizations. However, little studies and research investigated the chemical composition of the Syrian *Lepidium sativum*. Moreover, most of research work focused on the seeds of *Lepidium sativum* (chemical, biological and clinical studies), and not enough research work investigated the contents of the leaves. Therefore, the aim of this study is to examine the total phenolic content and the total flavonoids content as well as the evaluation of free radical scavenging properties of the *Lepidium sativum's* leaves compared to the seeds.

MATERIAL AND METHODS

Chemicals

Methanol , Chloroform (Panreac,EU), Ethanol, Aluminum chloride (Honeywell, Germany), Anhydrous Sodium Carbonate, Folin- Ciocalteu phenol reagent (Merck, Germany) Gallic acid, Ascorbic acid (Avonchem, United Kingdom), Quercetin (Sigma-Aldrich, Germany), 2,2-Diphenyl-1-picrylhydrazyl or DPPH(TCI Company, Japan).

Instruments

T80+UV- Vis Spectrophotometer (PG instruments, United kingdom), Hotplate Magnetic Stirrer (Snijders, Netherland), Rotary Evaporator (BÜCHI, Switzerland), Precision balance 180 A (Precisa, United Kingdom and Ireland).

Plant collection

The seeds were obtained from agricultural areas in Ghouta, Damascus, and then they were cultivated in November in the garden of medicinal plants, Faculty of Pharmacy, Damascus University. The leaves were harvested in February, while the seeds were collected in March and April. Plants were identified by Ph.D. Emad Al-Kady (PhD in botanical classification, faculty of Sciences, Damascus University, Damascus, Syria)*. The leaves and seeds were dried in the shade and they were stored in the opaque glasses containers at room temperature (20ºC).

- Due to the war in Syria most parts of the herbarium were lost. Therefore, we were unable to assess the number of this specific plant (the no of the voucher specimen of the plant).

Preparation of extracts

The methanolic and chloroformic extracts of the seeds and leaves were prepared by maceration method using magnetic stirrer with dissolving 20 g from dried powder of seeds and

leaves with 100 ml of each solvent (methanol 99.9% , chloroform) for 48 hrs. at room temperature (20ºC). The extracts were then filtered using filter paper. The solvents were removed using a rotary evaporator at 60ºC for methanol and 40ºC for chloroform. The extracts were dried until the weight was relatively constant. After that the crud extracts were stored in dark glassy containers at -20ºC until use. The process was repeated three times for each sample and yields were calculated.

Determination of total Phenolic content

Folin-Ciocalteu method was used to evaluate the Total phenol in methanolic and chloroformic extracts of the seeds and leaves according to Waterhouse[14]. A 100 µl of Folin-Ciocalteu reagent was mixed with a 20 µl of extract solution sample in test tube. Then a 1.58ml of distilled water was added with shaking and incubation in the shade for 9 min. at room temperature. Next a 300 µl of Sodium carbonate (20%) was added to the test tube that was kept in the shade for 45 min. The absorbance of all samples was measured at 765 nm with UV- visible spectrophotometer and the total phenolic content of extracts were expressed as mg of Gallic acid Equivalent (GAE) per 100 g of dried plant. (Gallic acid was used as standard).

Determination of total flavonoids content

The total flavonoids content of methanolic and chloroformic extracts (seeds and leaves) were determined by Aluminum chloride colorimetric method according to Abdel Kareem[15]. In the test tube, 1ml of Aluminum chloride (2%) was added to 1ml of sample then the tube was shaken and closed to incubate in the shade for 30 min. The absorbance of all samples was measured at 464 nm with UV-Visible spectrophotometer and using methanol as blank. The total flavonoids content each sample was expressed in mg of Quercetin equivalent per 100 g of dried plant. (Quercetin was used as standard)

Evaluating free radicals (DPPH radicals) scavenging activity

The antioxidant activity of methanolic and chloroformic extracts (seeds and leaves) was determined by evaluation DPPH radicals scavenging activity of extracts according to Al-Hussainy[16]. Different concentrations of each methanolic (50-150 µg\ml) and chloroformic extracts (50-3000 µg\ml) were prepared. A 1ml of DPPH reagent (0.1 mM) was added to a 3ml of each concentration of extracts. The mixture was shaken and incubated in the dark for 30 min. The decrease of absorbance of purple color (DPPH solution have purple color) was measured at 514 nm with UV- Visible spectrophotometer, methanol as blank and the methanol and DPPH reagent without extract as control. Ascorbic acid was used as standard. The free radical scavenging activity of all samples was expressed by calculating the inhibition percentage from following equation:

Inhibition percentage% = $(A_0 - A_1 / A_0)*100$

A_0: absorbance of Control,

A_1: absorbance of extracts

In addition to calculating The IC_{50} (the concentration of extract corresponding to inhibit 50% of free radicals {DPPH radicals}) for all samples. The reduction of IC_{50} indicated to the increase of free radical scavenging of extracts.

Statistical analysis

Results were expressed as the mean ± the standard deviation that were calculated from three replications. The data were analyzed by two ways ANOVA method to determinate significant differences between extracts using SPSS program (ver 26.0). The effect of the used part on the results was also determined by using one way ANOVA method.

RESULTS AND DISCUSSION

Results Yield of extraction

The yield of extraction were followed: 20.50% for methanolic extract of leaves, 19.13% for chloroformic extract of seeds, 17.22% for methanolic extract of seeds. The lowest yield of extraction was 2.50% for chloroformic extract of leaves.

Determination of total phenolic and total flavonoids contents

Total phenolic content of methanolic and chloroformic extracts from the leaves and seeds of *Lepidium sativum* were evaluated and calculated based on the linear - regression

equation of the calibration curve of Gallic acid used as standard (Figure1). Phenolic compounds of extracts are presented in (Table 1). The results showed that the total phenol content in seeds was higher than in leaves with respect to both methanolic and chloroformic extracts. Also, the methanolic seeds extract had the highest total phenolic compounds compared with the other extracts. However, the lowest total phenol content extract was chloroformic in leaves extract.

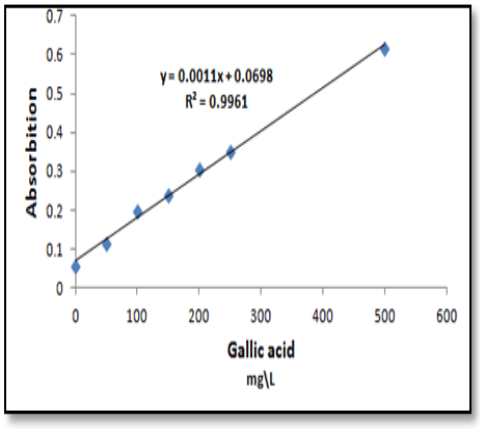

Fig. 1: Standard Curve of Gallic Acid

A similar analytical approach was used to determination of total flavonoids content of extracts (Figure 2). The results revealed that the leaves' extracts had the highest total flavonoids content compared to seeds' extracts (Table 1).

Table1: Total Phenolic content, total flavonoids content of ***Lepidium sativum*** seeds and leaves as plant material

Samples	Total Phenol Content (mg GAE\100g)	Total Flavonoids Content (mg QE\100g)
Methanolic Seeds Extract	1043.55 ± 0.15[a]	28.76 ± 0.29[a]
Methanolic Leaves Extract	993.43 ± 0.16[b]	153.32±0.60[b]
Chloroformic Seeds Extract	306.12 ± 0.12[c]	8.38 ± 0.01[c]
Chloroformic Leaves Extract	85.86 ± 0.01[d]	70.940.71[d]

- All Value s are calculated on dry plant material, GAE(Gallic acid equivalent), QE(Quersetin equivalent)
- The similar letters within same column indicated that is no significant s differences *(P< o.o5)*

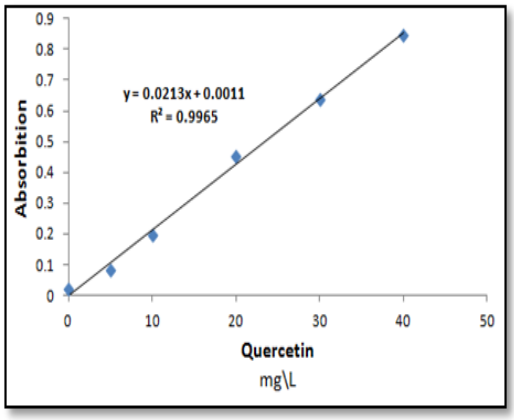

Fig. 2: Standard Curve of Quercetin

Evaluating free radicals (DPPH radicals) scavenging activity

Table 2 shows the values of IC_{50} (DPPH) for methanolic and chloroformic extracts from seeds and leaves.

Table (2): anti scavenging activity (DPPH assay) of ***Lepidium sativum*** Seeds and Leaves

Samples	IC_{50} (DPPH assay)
Ascorbic acid (VitC)	10.52 ± 0.09[a]
Methanolic seeds extract	50.97 ± 0.63[a]
Methanolic Leaves extract	126.43 ± 0.14[a]
Chloroformic Seeds extract	2988.07 ± 0.14[b]
Chloroformic Leaves extract	654.71± 0.05[c]

- IC50 the concentration of extract corresponding to inhibit 50 percent of free radicals{DPPH radicals]
- The similar letters within same column indicated that is no significant s differences (P< o.o5)

Disscusion

The results showed that the yield of methanolic extracts from leaves was the highest compared with the other extracts. This may be due to the difference of plant materials and the elevated content of polar compounds which dissolve in methanol more than chloroform according to Ghasemzadeh *et al.*[17].

The chloroformic extract of seeds was significant value of yield. This may be due to the high content lipophilic compounds in the seeds such as fattic acids, Vitamin E … etc according to Chatoui et al[18].

In this study, the results confirmed that the methanolic extracts of seeds and leaves had high concentration of phenolic compounds and flavonoids compared with the chloroformic with significant differences *(P< 0.05).* This may be due to methanol is more polarity than chloroform and the proper solvents to extraction the polyphenols which have various Phenolic and hydroxyl groups in their chemical structure, a few of these groups can form the glycoside bonds and combine with mono and polysaccharides to form Phenolic glycosides[19&20]. This structure makes the phenolic compounds more polarity[19].

The methanolic extracts from seeds had total phenolic content more than leaves. Such a result might be due to the content of phenolic compound in seeds is more than in leaves. Phenolic acids and their derivatives such as sinapic acid and its Choline derivative "sinapin" are the common phenolic compounds in *Lepidium sativum* seeds depending on Al-Snafi[21]. This result was consistent with the reported results from Koli et al study which confirmed that the seeds had a total phenol content of (8.53 ± 0.321 mgGAE\g plant material)[22].

In addition, the results revealed that the phenolic content was higher in chloroformic extracts of the seeds compared to chloroformic extracts of the leaves by a significant different levels *(P< 0.05).* This increase in seeds may be due to the presence of a few derivatives of phenolic acids which contains lipophillic substitutes (such as fatty acid ester or cyclic lactones, etc) more than in leaves according to Liu, *et al.*[23].

The results showed that leaves extracts had statistically different higher levels of flavonoids content than seeds' extract (*P< 0.05*). This indicates that leaves contains higher flavonoids (in methanolic extract) compared to the seeds. This was consistent with Mojzer, et al who found that the flavonoids concentrate particularly in the aerial part of the plant[20]. Also, these results agree with Agarwal, *et al.* who isolated three compounds of flavonol glycosides from alcoholic aerial parts extract of *Lepidium sativum*[24]. The chloroformic leaves' extract has exhibited significant elevating in the results, it may be due to leaves might contain free flavonoids (as aglycons) more than seeds. Although chloroform is the slight polarity solvents compared to methanol, chloroform has been used to extract and evaluate some types of flavonoids such as flavones, flavanones, and methylated flavonoids[19]. Moreover, the residues of chlorophyll in chloroformic extract interacted with absorption of samples in analysis and caused the increase in the measured levels of Total content flavonoids according to Abdulkadir *et al.* [25].

The results also showed that the highest inhibition activity of free radicals extract was the methanolic extracts of t seeds and leaves', followed by the chloroformic extract of leaves and the least inhibition activity was due to the chloroformic extract of seeds compared to Ascorbic acid. This can be explained by the methanolic seeds' and extracts of leaves had the highest concentration of phenolic compounds compared to other concentration (Table 1). The increase of phenolic content lead to an increase in inhibition activity in methanolic extracts (Figure 3) . The chemical structure of phenolic compounds is rich in hydroxyl groups linking to aromatic rings which could donate hydrogen atoms to free radicals and become more safe and stable compounds for abolition free radicals[26]. In addition, this type of phenolic and flavonoids compounds had most antioxidant activity because of their chemical structure according to Minatel *et al.*[19]. The antioxidant activity of the phenolic compounds and flavonoids depends on the number and position of hydroxyl groups in the aromatic ring (presence of hydrogen atoms on ortho position for hydroxyl groups in aromatic ring increased the inhibition activity). Moreover, the double bonds and oxo groups in the aromatic ring improved the free radicals scavenging of the moleculars[19].

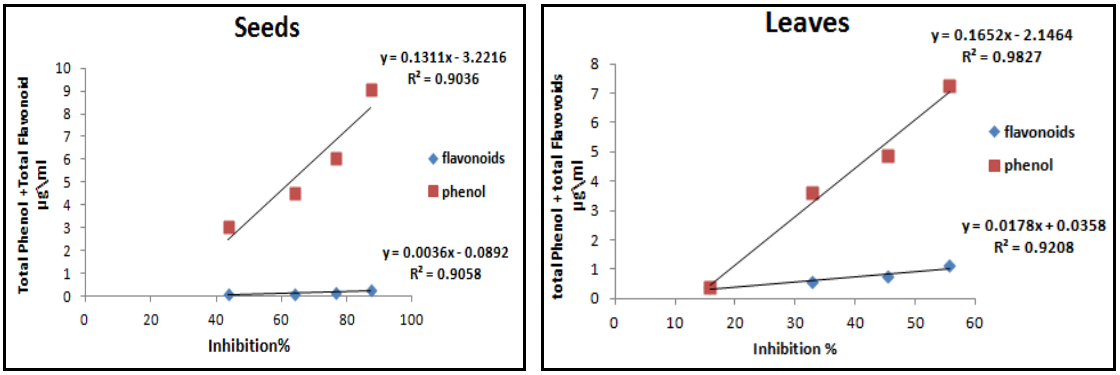

Fig. 3: Correlation between the phenolic content, flavonoids content and free radicals scavenging activity in the Leaves and seeds of *Lepidium sativum*

The results of antioxidant activity of the methanolic extract of seeds and leaves extracts respectively were higher than results found by Arunachalam et al, who reported that free radicals inhibition in 100µg\ml of ethanolic extracts of seeds and leaves were 11.63 ± 0.3% and 10.21 ± 0.7% respectively. The difference may be due to change in the extraction method (soxhelt apparatus is used without relating to duration time of extraction which may be not enough to extract antioxidant compounds successfully). Moreover, the difference of plant origin (India)[27].

The chloroformic seeds extract had gave lower value of inhibition activity and higher value of IC_{50} compared with ascorbic acid and other extracts ($p< 0.05$). This was disagreed with Chatoui et al, who reported that seeds contains lipophillic antioxidants such as vitamin E, omega 3-fatty acids[18]. Such a discrepancy is due to the DPPH assay was not suitable to evaluation the antioxidant activity for lipophillic extract as reported by Zhu [26]. Also, these results are lower than those reported by Indumathy and Aruna who showed that the antioxidant activity of the chloroformic seeds extract was IC_{50}= 1165 µg\ml. Such a difference in the results may be due to variation of environmental conditions, plant origin (India) and extraction method (by using Soxhlet apparatus for 24 hrs. as the high temperature in Soxhlet apparatus improve the yield extraction)[28].

Conclusion

Lepidium sativum is popular plant in the world, especially in Syria and the Middle East. This research study examined the chemical extracts from the leaves and seeds of *Lepidium sativum*. It has been confirmed that leaves have phenolic content lower than seeds. However, the Flavonoids content of Leaves was superior to the flavonoids content of seeds. Moreover, the leaves had antioxidant activity in addition to the antioxidant activity of seeds. This indicated that the leaves contain flavonoids compounds may have important therapeutical effects and impressive physiological activities such as anti-inflammatory activity, antioxidant and anticancer and can combine nutritional and therapeutic benefits. These results show that *Lepidium sativum* leaves as well as seeds can be considered as a natural antioxidant and nutritional supplements without undesirable side effects compared to the chemical medication.

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تقييم المحتوى الفينولي الكلي ومحتوى الفلافونوئيدات بالإضافة لتقييم الفعالية المضادة للأكسدة لبذور وأوراق نبات الرشاد LEPIDIUM SATIVUM L. المزروع في سورية

نجوى مهند سلطان* – رشا خطيب

قسم العقاقير ، كلية الصيدلة ، جامعة دمشق ، سورية

اهتمت العديد من الأبحاث بدراسة بذور نبات الرشاد Lepidium sativum L. دراسة كيميائية وسريرية في حين لم توجد دراسات كافية اهتمت بالتركيب الكيميائي لأوراق النبات. في هذه الدراسة، تم تحديد المحتوى الفينولي الكلي ومحتوى الفلافونوئيدات الكلية باستخدام للخلاصات الميثانولية والكلوروفورمية لكل من بذور وأوراق نبات الرشاد. بالإضافة لتقييم الفعالية المضادة للأكسدة بتقييم الفعالية الكاسحة للجذور الحرة للخلاصات السابقة الذكر باستخدام طريقة DPPh. أظهرت النتائج بأن البذور تمتلك محتوى فينولي(خلاصة ميثانولية: ١٠٤٣±٠.١٦ملي جرام GAE/١٠٠جرام ، خلاصة كلوروفورمية: ٣٠٦.١٢±٠.١٢ملي جرام GAE/١٠٠جرام) أعلى من المحتوى الفينولي للأوراق (خلاصة الميثانولية: ٩٩٣.٤٠±٠.١٦ملي جرام GAE/١٠٠جرام ، خلاصة كلوروفورمية: ٨٥.٨٦±٠.٠١ملي جرام GAE/١٠٠جرام) في حين إن محتوى الفلافونوئيدات في الأوراق (خلاصة ميثانولية: ١٥٣.٣٢±٠.٦٠ملي جرام GAE/١٠٠جرام ، خلاصة كلوروفورمية: ٧٠.٩٤±٠.٧١ملي جرام GAE/١٠٠جرام) أعلى منها في البذور (خلاصة ميثانولية: نبات جاف ٢٨.٧٦±٠.٠١ملي جرام GAE/١٠٠جرام ، خلاصة كلوروفورمية: نبات جاف٨.٣٨±٠.١٢ملي جرام GAE/١٠٠جرام). علاوة على ذلك تبين بأن كل من الأوراق (خلاصة ميثانولية: IC50= ١٢٦.٤٤±٠.٦٣ميكروجرام/مول ، خلاصة كلوروفورمية: IC50 = ٦٥٤.٧١±٠.٠٥ميكروجرام/مول) والبذور (خلاصة ميثانولية: IC50=٥٠.٩٧±٠.١٤) تمتلك فعالية مضادة للأكسدة . وتبعا للنتائج السابقة يمكن أن يعتبر نبات الرشاد مصدر طبيعي للمركبات المضادة للأكسدة ومكملات غذائية مهمة .