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IN-VITRO EVALUATION OF THE ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIBACTERIAL ACTIVITY OF *LAMIUM ALBUM* EXTRACTS

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ABSTRACT: *Lamium album* is used as a blood purifier, in the treatment of the respiratory tract diseases, diarrhea and bleeding. In this study antioxidants and anti-bacterial activities of its aerial parts and roots were investigated. Extracts were prepared by Soxhlet apparatus. Methanol used as extracting solvent. Phenol and flavonoids contents and antioxidant properties were evaluated by different methods. Different concentrations of extracts were used for determining the MIC. Microorganisms suspensions were prepared in Mueller Hinton broth with different concentrations of extracts and incubated for 24 h at 37 °C, the turbidity of the tubes were observed. MIC and MBC were determined. Total phenolic content of aerial and roots extracts were 242.75 ± 10.13 and 135.0 ± 8.15 and total flavonoid contents were 79.83 ± 4.22 and 30.33 ± 1.08 QE respectively. The DPPH radical-scavenging ability of extracts were 238.4 and 257.0 µg/ml respectively. Reducing power of both extracts increased by increasing the concentrations. The amount of scavenging nitric oxide at the 1600 mg/ml, was 58 and 68%, respectively. IC_{50} for chelating activities of extracts were 1139 and 1323 µg/ml for aerial and root extracts, respectively. Antimicrobial property of the aerial parts against *E. coli* in microdilution method was better than root extract. Its mean diameter of inhibition was 17 mm. On *Klebsiella* the activity on the root extract was better. Its inhibition diameter was 11.66 mm. In conclusion, the anti-microbial and antioxidant activities of *L. album* aerial parts was higher than root extract.

INTRODUCTION: Medicinal plants as the natural source of medicine have been used since ancient times. Chemical products besides certain advantages, have some disadvantages which approach makes use of natural products in the field of medicine, nutrition and the industry.

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Today, extensive research on medicinal plants in order to identify active ingredients, properties and pharmacological activities are carried [1]. Herbal treatments include features such as availability and performance. They are also mentioned in cultures, old books, and divine religions [2, 3]. The role of free radicals in the pathogenesis of many diseases has been established. Many biochemical reactions in the body produce reactive oxygen molecules that have biological degradation capacity. The harmful effects of free radicals, could be blocked by anti-oxidant. Scavenging of free radicals, could cause detoxification.

Foods rich in antioxidants play an essential role in the prevention of cardiovascular disease, cancer, degenerative diseases (Parkinson's and Alzheimer's). Flavonoids are widely present in food, fruits and vegetables and many of them have anti-cancer property. These compounds are present in the extracts of these plants [4]. Some plants contain phenolic compounds with antioxidant properties that are closely related. Plants that are rich in antioxidants may protect cells from oxidative stress. Activated phagocyte ingagents to destroy invading bacteria and fungus need to use these compounds. Superoxide have useful role in regulating cell growth and intercellular messages. Plants with antioxidant properties can also have anti-inflammatory, anti-depressant, and so on effects [5].

In recent years, much research has been done to find effective compounds against bacterial fungal and parasitic infections [6]. Control of micro-organisms in the environment and in preparing various materials intended for human consumption is important. *Klebsiella pneumoniae* has polysaccharide capsule which play important role against host defense. *Escherichia coli* a facultative, non-sporeforming bacteria ferment glucose and produces gas [7], can destroy many microorganisms and inhibit growth of many other organisms. Medicinal plants products have antimicrobial properties with fewer side effect than the synthetic products. Also they have other medicinal properties too [8]. Recently plant products such as, extracts and essential oils extracted have been studied for their antimicrobial properties [9]. It was cleared that some of them act against parasites, fungi, viruses and bacteria [10]. *Lamium album* (in Persian white nettle) is from family of Lamiaceae. North (Gilan and Mazandran provinces) and northwest of Iran is the natural habitat of this plant **Fig. 1** [11].

FIG. 1: *LAMIUM ALBUM*

This plant grows in moist, shaded areas of the forest edge in Europe, Asia and North Africa. Seven species of this genus grow in Iran. The most important active ingredients of this plants are tanins, saponins, mucilage, volatile oil, potassium, flavonoids, glycoside isokercitine, tyramine, histamine and choline. It is somewhat similar to ordinary nettle (*Urticadioica*), but is actually different. Some of *Lamium* species, around the world, traditionally are used to treat injuries, fractures, infections, high blood pressure; also as a blood purifier, healer, diuretic, narcotic [12, 13]. *L. album* has been used in raw or cooked or tea for a long time, especially in the Mediterranean region. Evidence indicate its anti-inflammatory and antioxidant properties [14, 15]. From the aerial organs compound such as colin, camfrol and camfrol-3-glucoside are found and leaves are eatable which are rich source of carotene.

In India, its flowers used to stop bleeding, help sleeping, as a blood purifier and in treatment of hemorrhoids bleeding. In Spain its root is used in wound healing and its flower to eliminate severe diarrhea. Studies on the *L. album* aerial parts extract in order to evaluate its pharmacological properties have been done and showed the following properties in different condition: purify the blood, lowers blood sugar, treats anemia and mild diarrhea, stomachulcers, kidneystones, rheumatism, varicoseveins, sore, insomnia, dandruff, depression, regulate menstruation, induce hair growth, reduce joints pain and detoxifies the body [5]. Other effects are in preventing menstrual disorders, abdominal inflammation, musculo-skeletal diseases [15] antioxidant properties [16]. Its leaves have antibacterial property [17]. This study aimed to evaluate the antioxidant activity, total phenolic and flavonoid contents and assess the antimicrobial properties of different organs.

MATERIALS AND METHODS:

Plant Extracts: *Lamium album* L. samples were collected in spring from the natural habitats of Sari city (Gale Kola Sofla Kordkheyl forest villages). The herbarium of each sample was prepared and kept in the Payamnoor University of sari branch herbarium center (voucher specimen 35-93). Flowering aerial parts and roots of plants were dried in the shade. Samples were ground. 30 g of powder was extracted by Soxhlet apparatus using

methanol as solvent for 8 h. The solvent was rotary evaporated in vacuum then dried using freeze-dryer [18].

Flavonoids Measurement: Total flavonoids were estimated using the method of our recently published paper [19]. Briefly, 0.5 ml solution of extract in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve. Contents were expressed as quercetin equivalent (QE).

Total Phenolic Content: Total phenolic compound contents were determined by the Folin-Ciocalteau method according to the recently published method [19]. The extracts samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Experiment was repeated three times and mean was reported.

Determination of Reducing Power: Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. The Fe^{3+}- reducing power of the extract was determined according to our recently publish paper [20]. Different amounts of extracts (50-1600 µg ml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%).

The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm against an appropriate blank solution. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control. All tests were performed triplicate.

DPPH Radical Scavenging Activity: The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical scavenging activity of the extracts [20]. Different concentrations of extracts were added, at an equal volume, to methanol solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C and BHA were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Iron II Chelating Efficiency: The ability of the extracts to chelate ferrous ions was estimated by our recently published paper [21]. Briefly, the extracts (0.2-3.2 mg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine- Fe^{2+} complex formation was calculated as $[(A_0 - A_s)/As] \times 100$, where A_0 was the absorbance of the control, and As was the absorbance of the extract / standard. Na_2 EDTA was used as positive control.

Nitric Oxide Radicals Scavenging Efficiency: The ability extracts for scavenging nitric oxide were estimated according to our recently published paper [21]. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of extracts dissolved in water and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control [21].

Antimicrobial Effects: Concentrations of 25, 37.5, 50, 75, 100 and 150 mg/ml in 10% DMSO (Dimethyl sulfoxide (aminsan/Iran) was prepared and used to determine the MIC (minimum inhibitory concentration) and Disc diffusion. Microorganisms under study were lyophilized *Klebsielle* (ATCC 7881) and *Escherichia coli* (25922 ATCC) provided from Tehran University culture collection. 4-5 colonies of the young culture was inoculated to the sterile Mueller-Hinton broth (*Fluka*).

Turbidity of microbial suspensions prepared in accordance with the 0.5 McFarland standard (turbidity of 1.5×10^8 bacteria/ml) were tested. The samples were diluted. Antimicrobial activity of extracts were examined by Agar Well Diffusion and Microdilution methods. In the Agar well method, the suspension with concentration of 1.5×10^6 cfu/ml was cultured on the medium in three directions, then the different concentrations of extracts were added in the well. The negative and the positive control were the solution used for solving the extract (10% DMSO) and gentamicin (*Caspian tamin*), respectively. The plates were incubated for 24 h at room temperature and after the formation of microbial growth, zone of inhibition was measured in millimeters [22].

Using the dilution method, minimal inhibitory concentration and minimal bactericidal concentration of methanol extracts were determined. Eight sterile tubes were selected and 0.5 ml of sterile Mueller-Hinton broth added to the tubes, Then 10 mg of bacterial suspension was added to 10 µg of different concentrations of extracts (25, 37.5, 75, 100 and 150 mg/ml). In the other tube, saline was added instead of extract. The positive control was considered bacteria without extract and negative control bacteria with extract. The last tube with no turbidity (no growth) was considered as the MIC.

All the tubes with no turbidity were cultured onto the plate medium, incubated at 37 and for 24 h to determine the MBC (minimum bactericidal concentration). The plate with no growth corresponding to the lowest concentration of the tube was considered as the MBC. Concentration with counting the number of colonies at 99.9% was determined. The test was repeated for 3 times. The obtained data were analyzed by ANOVA, analysis of variance and chi-square. The significance of difference was determined at the level of $p<0.001$ [23].

RESULTS:

Extracts Yields: The yield of aerial parts and root were 19 and 11%, respectively.

Data Obtained on Antioxidant Property: Assessment of the Phenolic Content: To assess the total phenolic content of the extracts the Folin-Cyoculto was used. Calibration curve was plotted according to Gallic acid standard and the resulting equation was calculated as follows: y = 0.005x + 0.026 (correlation coefficient 0.997). The total phenolic content of the aerial parts and root were 242.75 ± 10.13 and 135.0 ± 8.15 GAE mg/g of extract.

Determination of Flavonoids Contents: The total flavonoid content of extracts was measured according to the colorimetric method. Quercetin was used as standard. After plotting the standard curve, the equation line was obtained as follow y= 0.006x (correlation coefficient 0.998)

The total flavonoid content of extract of aerial parts and root were 79.83 ± 4.22 and 30.33 ± 1.08 respectively.

The Scavenging Rate of DPPH Radical: The IC_{50} for standards and the extracts were obtained. For BHA it was 53.9 µg/ml and for ascorbic acid 5.05 µg/ml; for aerial parts and root extracts 238.4 and 257.0 µg/ml, respectively. The radical-scavenging efficiency in all extracts increased with increasing of concentrations.

Reducing Efficiency: Extracts in concentrations of 25 to 800 µg/ml had poor reducing power. Insignificant differences were observed among the extracts reducing power ($p>0.05$) but this difference was statistically significant compared with vitamin C ($p<0.01$) **Fig. 2**.

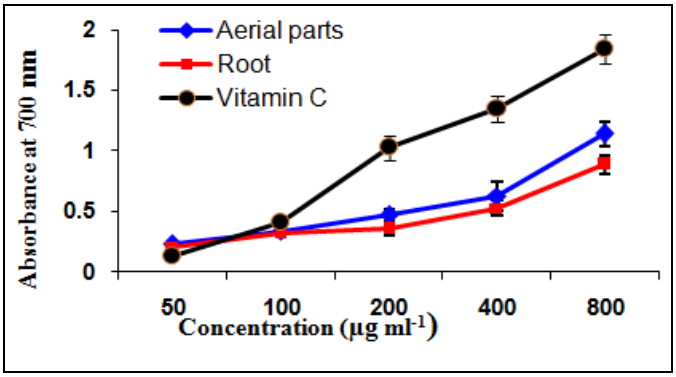

FIG. 2: THE REDUCING EFFICIENCY OF THE AERIAL AND UNDERGROUND ORGANS EXTRACTS IN THE *LAMIUM ALBUM* VITAMIN C WAS USED AS POSITIVE CONTROL

The Rate of Nitric Oxide Scavenging Efficiency: The IC_{50} for *L. album* aerial aerial parts extract at the highest concentration, 1600 mg/ml, was 58%. The root extract at the same concentration showed a 68% inhibition. The IC_{50} for quercetin (used as

positive control) was 37.9 mg/ml. The extracts acted much weaker than quercetin ($p<0.01$).

The Iron Chelating Property: IC_{50} values for the aerial and root extracts were 1.13 and 1.32 g/ml, respectively. Both extracts showed weak chelating efficiency. IC_{50} for EDTA chelating efficiency was 17.5 µg/ml. Antimicrobial the findings:

Antimicrobial the Findings: The MIC for methanol extract of *L. album* aerial parts on *E. coli* was 100 mg/ml and MBC value was 150 mg/ml **Table 1**. Micro dilution method confirmed the well method. The antimicrobial activity of the aerial parts extract was better than that of the root extracts **Fig. 3**. The MIC of *L. album* aerial parts extract at concentration of 150 mg/ml on *Klebsiella* was better than root extract. Similar data was found in the well method. The MIC of aerial parts on *Klebsiella* was 150 mg/ml and the MBC was observed at the higher concentration **Table 1**.

TABLE 1: MEAN OF ZONE OF INHIBITION IN THE STANDARD SPECIES UNDER STUDY AGAINST DIFFERENT CONCENTRATIONS OF THE *LAMIUM ALBUM* AERIAL ORGAN EXTRACT

The standard species	Concentration of every extract of *Lamium album* (mg/ml)						
	150	100	75	50	37.5	25	P-value
E. coli	16.33 ± 2.08	17 ± 2.645	15 ± 2.64	11.66 ± 1.52	9.33 ± 1.52	8.33 ± 0.57	0.022
klebsiella	10.66 ± 0.96	10.33 ± 0.89	7.33 ± 1.06	3.6 ± 1.25	1.3 ± 0.99	0 ± 0.98	0.0095

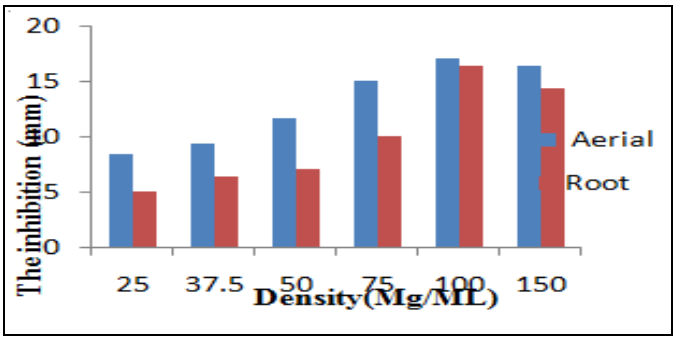

FIG. 3: ZONE OF INHIBITION OF THE AERIAL AND UNDERGROUND ORGANS EXTRACTS OF THE *LAMIUM ALBUM* ON *E. COLI*

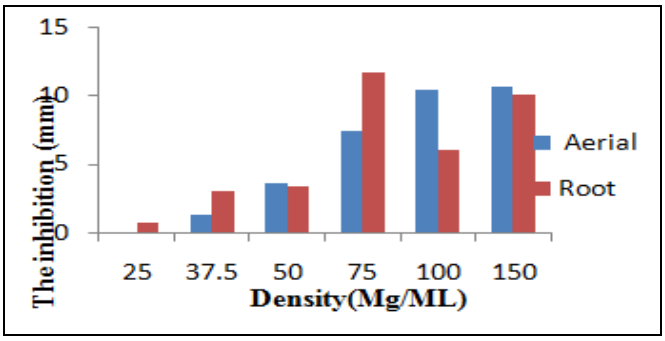

FIG. 4: ZONE OF INHIBITION OF THE AERIAL AND UNDERGROUND ORGANS EXTRACTS OF THE *LAMIUM ALBUMON KLEBSIELAE*

MIC at the concentration of 75 mg/ml was better on *Klebsiella* and similar result was observed in well method. The MBC was observed at 100 mg/ml **Table 2**. The antimicrobial activity of root extract was better than the aerial parts extract. Overall, evaluation of the antimicrobial activity between the aerial and root extracts of *L. album* on *Klebsiella*, indicated that the result of micro dilution method is confirmed by well method. The aerial parts extract at concentration of 150 mg/ml and the root extract at concentration of 75 mg/ml were better. In all, the antimicrobial effect of the root extract was better than the aerial parts extract. The results of the effect of methanol extract of *L. album* in the disc diffusion method on different species of microbes are given in the **Fig. 4**.

The effect of different concentrations of the aerial and root extracts of *L. album* on the growth inhibiting diameter of *Klebsiella* and *E. coli* revealed significant difference ($p\leq0.05$). Based on statistical analysis by ANOVA, the zone growth inhibition at high concentrations was more than low concentrations. The extracts had better antimicrobial effect on the *E. coli* which could be due to presence of two cell membranes in the gram negative bacteria. The results of effect of root extract on *E. coli* by microdilution method showed that the concentration of 100 mg/ml will act better. Similar result was obtained with the well method. The MIC of root extract on *E. coli* was 100 mg/ml and MBC value was 150 mg/ml **Table 2**.

TABLE 2: MEAN OF ZONE OF INHIBITION IN THE STANDARD SPECIES UNDER STUDY AGAINST DIFFERENT CONCENTRATIONS OF THE *LAMIUM ALBUM* UNDERGROUND EXTRACT

The standard species	Concentration of every extract of *Lamium album* (mg/ml)						
	150	100	75	50	37.5	25	P-value
E. coli	14.33 ± 1.15	16.33 ± 0.57	10 ± 2.64	7 ± 1.0	6.33 ± 2.51	5 ± 1	0.022
klebsiella	10 ± 0.89	6 ±0.53	11.66 ±1.25	3.33 ±1.32	3 ±1.55	0.66 ±0.99	0.0095

DISCUSSION: In this project we examined the antioxidant and antibacterial activity *L. album*. Antibacterial property of Compositae and Lamiaceae plants essential oils were studied against nine strains of gram-negative bacteria and 6 strains of gram-positive bacteria [24]. Cetin *et al*, (2006) studied the cytotoxic activity of extracts of *Labiatae* (Lamiaceae) on the larvae. The aerial organ ethanolic extract of 5 species of the family of *Labiatae* was obtained from Turkey to assess the cytotoxic activity against mosquitoe larvae *Culex pipiens* [25].

Erdemoglu *et al.,* (2006) investigated the aerial organ antioxidant effect of 4 plants from the family of *Lamiaceae*, using DPPH as well as the FIA-CL. All the extracts showed significant effect against DPPH free radicals and inhibitory effect on H_2O_2 or HOCl luminolchemiluminescence. These extracts inactivated 50% of DPPH radical in the following descending order: *Stachys byzantina*, *Salvia viridis*, *Salvi amulticaulis*, *Eremostachysl aciniata*. The strongest extract of H_2O_2-induction, belonged to *Salvia viridis* and HOCl induction belonged to *Stachys byzantine* extract. The results showed that these natural extracts are potential antioxidant [26].

Paduch *et al.,* (2008) studied the growth inhibition and cytotoxic effect of free radical of *L. album*. The methanolic extracts are mainly rich in flavonoids and phenolic acids and the ethyl acetate extract toxic effect against normal fibroblasts plastic (HSF). The obtained data indicate that the extract under study has potential benefits in preparing of natural formulations [27]. Zolfagari *et al.,* (2012) in their study in Arasbaran (Iran) on medicinal plants, evaluated the pharmacological properties of *L. album* flowering branches, and found the anti-inflammatory and diuretic effects, being healer, useful in treatment of respiratory tract and spleen diseases.

In flowers, fruit and other plant tissues, the effective chemical compositions such as tannins, mucilage, sugar, glycoside and saponins were reported [13]. Nemati *et al.*, (2012) in Kermanshah province (Iran) in their studies on the medicinal plants, identified that the aerial part and roots of the *L. album* L. has medicinal properties [28]. Bubueanu *et al.*, (2013) reported the significant antioxidant property in butanol extract of *lamium album* and *L. purpureum* by DPPH and chemiluminescence methods [29].

The Total Phenolic and Flavonoid Content: The mechanism of action of flavonoids as antioxidant is through collection of free radicals such as superoxide anion and lipid peroxide and free hydroxyl radicals, besides the efficiency to scavenge single oxygen and chelation of metals as well. The antioxidant property of this class of materials is pertaining to the presence of aromatic groups and hydroxyl free radical. Flavonoids with freer hydroxyl radicals do better scavenging of the radicals [30]. Extract of aerial organ has more phenolic and flavonoid content than the root extract.

The total phenolic content of aerial and root extract of *L. album* is more than *Diospyroslotus*, *Sambucus ebulus*, *Ferula assafoetid*a, *Grammoscia diumplatycarpum*, *Feijoa sellowiana*, *Pterocarya fraxinifolia* and wild pear (*Pyrus boissieriana*) and the root extract has less total phenolic content than *Melilotus arvensis*, *Artemisia absinthium* L, *Parrotia persica Mey* and *Salvia glutinosa*. The plant flavonoid content in the aerial and the root of *L. album* extract is higher than *Alcea hyrcana Grossh*, *Hyoscyamus squarrosus*, *Colchicum speciosum Steven*, *Zea mays*, *P. fraxinifolia*, *P. boissieriana* and *A. absinthium* L. also total flavonoid content in root of *L. album* is lower than the *S. glutinosa* and *M. arvensis* [20, 30, 31] as well as in plants such as *Achillea wilhemsii* [18], plant *L. album* and *Hypericum perforatum* L. [32] than the DPPH, scavenging of nitric oxide, iron chelating activity was weaker, and the reducing power is substantial and the phenol is better than the yarrow plant have been weaker than *H. perforatum* L. The plant flavonoids and *L. album* has been better than *H. perforatum* L.

The Scavenging Efficiency of DPPH Radical: DPPH radical is a stable free radical with the central nitrogen atom, When reduced, is converted to a stable DPPH-H molecule and turn from purple to yellow color. DPPH radical absorbance is at 517 nm, but as soon as reduced by an antioxidant, the absorption is reduced. The antioxidant activity of samples is expressed by disappearance of purple color [20].

To evaluate the scavenging rate of DPPH, DPPH color was used as an indicator. On this basis, the more efficiency in scavenging the radicals, the more intensity of the purple decline to yellow color. The IC_{50} for standards and the extracts were obtained as follow: the IC_{50} for BHA 53.9 µg/ml, ascorbic acid 5.05 for µg/ml, for *L. album* aerial parts extract 238.4 µg/ml for and root extract 257.0 µg/ml. Radical-scavenging activity in all extracts increased by increasing the concentration of the extract, but the aerial organ was more active. By comparing the IC_{50} of this plant with some species of this plant in the north of Iran, the anti-oxidative activity of them could be reviewed. The fruit methanol extract of *D. lotus* and *Pyrus boissieriana* showed the IC_{50} value of 1450 and 3000, respectively. The leaf methanol extract of *Eryngium caucasicum* and fruit of *Crataegus elbursensis* extract had IC_{50} values of 270 and 341.29, respectively.

The Reducing Power: Reducing power, indicates the electron donating efficiency of antioxidants. If a substance having this property could reduce the amount of the oxidized intermediate made during the lipid peroxidation. Therefore would break the reaction chain and acts as primary and secondary antioxidants [20]. Measurement of the reducing power of sample was performed by reducing iron III (ferric ion) to iron II (ferrous ion). Due to reduction a particular blue color named Prussian blue could develop at wavelength of 700 nm. The rate of iron complex could be determined by measuring the formation of Prussian blue. Increase of absorption at this wavelength is indicative of the absorption capabilities. In this study, it was found that, the reduction power of both extracts increased with increasing of the concentrations, but these extracts as compare with ascorbic acid showed lower rate of reduction.

The Nitric Oxide Scavenging Rate: Nitric oxide is free radical with a single electron, is produced from L-arginine. Nitric oxide scavenging is in competition with oxygen, causes a reduction of nitrite ion. Therefore, the more scavenging, the less nitrate production and less absorption, hence less color could develop [32]. The IC_{50} levels for the standard quercetin was obtained 37.9 mg /ml. For the aerial organ extract of *L. album* that tested at the highest concentration was 1600 mg/ml, with 58% inhibition and of root extract at the same concentration was 68% inhibition. The extracts acted much weaker than quercetin.

The Metal Chelating Efficiency: Most food in contact with the metal ions undergoes oxidation reactions. These metals are present in food or enter the food during processing. Bivalent intermediate metals, iron, the most common transfer electrons, through the Fenton reaction, produces a large amount of hydroxyl radicals. In this way, the damaging effects of hydro peroxide increases. The amount of hydroxyl radical which is the most important free radicals in biological tissues could be determined. EDTA, ascorbic acid, citric acid and phenolic compounds by chelating the metals, such as iron prevent the production of hydroxyl radicals. Prevention of harmful reactions such as Fenton is important in human health and shelf-life of food, cosmetic and pharmaceutical products [18].

In this study, Frozin detector was used which makes a red complex with iron II. The iron concentration in the presence of chelating agents is reduced and the red color of iron-Frrozin complex declines. In this test, EDTA was chosen as control. Both extracts showed poor chelating effect. The fruit methanol extract of *D. lotus* and *Pyrus boissieriana*, had acceptable values of IC_{50} and with stronger effect than the *L. album.* In all, all of the antioxidant activities of aerial organ of *L. album* was better than the root extract in all of the tests performed, but the root extract was stronger in scavenging nitric oxide. The aerial parts extract had higher activity can be attributed to higher phenol and flavonoid content.

Antimicrobial Activity: In the present study using the agar diffusion susceptibility testing showed significant antimicrobial activity of plant extracts against microorganism's. The obtained data were consistent with results of MIC testing. The range of MIC of extract against the two standard species were 100 to more than 150 mg/ml, and 75 to more than 37.5, 25 and 50 mg/ml. respectively. These extracts at different concentrations had anti-microbial activity. *L. album* extract at the highest concentration showed antimicrobial activity against Gram-negative bacteria. In this regard, this study aimed to evaluate the antimicrobial activity of alcoholic extracts of aerial and root extract of *L.*

album against *E. coli* and *Klebsiella* species. In all, aerial parts extract had higher inhibitory effects than the root extract against the testing standard microbes. Both aerial and root extracts had the most concentration-dependent inhibitory effect against *E. coli* and *Klebsiella*. Both extracts under study at concentrations of 25 mg/ml and 100 mg/ml had the lowest and the highest antimicrobial activity, respectively. The different concentrations of the root and aerial organ of *L. album* extracts, in general, high concentrations (150 mg/ml) to low concentration (25 mg/ml) we observed orderly decrease in the diameter of zone inhibition, orderly.

Today many studies have been done to determine the antimicrobial activity of the hydro extract and the organic substances of the medicinal plants on the *E. coli* and *Klebsiella*. Gulluce *et al.*, in their study, found *E. coli* sensitive to the methanol extract of the European oak leaves with a concentration of 300 µg/ml [33]. Chipeva and colleagues (2013) in their study on antimicrobial activity of aqueous extract of *L. album* found that the leaves extract has higher antimicrobial activity than the flower extract, and could be used antimicrobial agent [17]. Davoodi *et al.*, (2017) studied on the antibacterial activity of *Mespilus germanica* leaf extract, results showed best inhibitory and bactericidal activities against *Klebsiella pneumonia* (MIC= 3.333 ± 0.0233 and MBC= 5.833 ± 0.065). The lowest MIC was observed against *Vibrio cholera* (6.667 ± 0.048) and the lowest MBC was seen against *E. coli* and *Shigella dysentiea* (9.167 ± 0.042) [34].

Although the study was Antibacterial effect of hydroalcoholic extract of *Astragalus hamosus* done on *E. coli*, and zone of inhibition at well method at concentrations of 50, 100, 200 and 400 mg/ml was 10, 12, 16 and 18 mm, respectively [35].

CONCLUSION: In the present study, though it was done at high concentration of 150 mg/ml on Gram-negative bacteria, but the mean concentrations were 75, 100, 150 and 50 mg/ml and zone of inhibition were 16.33, 17, 15 and 11.66 mm respectively. Differences in antimicrobial effects of different parts of the plant could be due to presence of different amount of tanan and the other effective substances. Tanans have various properties including antimicrobial effects. Though extracts of this plant had total phenolic and flavonoid content.

In our study we found the antibacterial properties of methanol extract of aerial and root organs extract of *L. album* on *klebsiella* and the aerial organ had more antibacterial property. Total phenolic content and antioxidant effects have been reported in the *L. album* plant which is relatively higher in the aerial organ and also flavonoid content was present more in the aerial organ. In the higher concentration the effect was more. Alkaloids which have anti-microbial activity have been identified in the *L. album*. Alkaloids content are more reported in the family of Lamiaceae. The alkaloids are present in all organs of the plant. Other important compounds such as tannins, mucilage, volatile oil, potassium and flavonoids, glycosides and histamine has long been in folk medicine as a medicinal species.

In this study the antimicrobial properties of the aerial organ extract was more on *E. coli* but the extract had more effect on the *Klebsielae*. In all, the aerial organ extract had higher antimicrobial activity by micro dilution and well methods.

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