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ANTIOXIDANT CAPACITY, CHROMATOGRAPHIC ANALYSIS OF PHENOLIC COMPOUNDS AND ANTI-MICROBIAL EFFECTS OF *CARDARIA DRABA* GROWING IN IRAN

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ABSTRACT: In the present study, the antioxidant and anti-microbial potential of aerial and underground parts of the *Cardaria draba* (L.) Desv. Different tests evaluated methanolic extracts. The phenol and flavonoid contents of the extracts were investigated by Folin Ciocalteu and $AlCl_3$ assays. Phenolic compounds analyzed By HPLC method. DPPH Radical-Scavenging, reducing power, nitric oxide-scavenging, and Fe^{2+} chelating tests were used to measure antioxidant activity and evaluate anti-microbial properties MIC, MBC, and ZOI values of extracts were measured. Extracts contained significant amounts of phenols and flavonoids. In the HPLC analysis, chlorogenic and a suitable mobile phase separated coumaric acid peaks. IC_{50} of DPPH Radical-Scavenging Activity for the aerial part was 403 ± 2.3 µg ml^{-1} and for the underground part was 555 ± 3.1 µg ml^{-1}. Extracts showed weak reducing power that significantly differed significantly with Vitamin C ($p < 0.01$). In the case of the nitric oxide-scavenging effect, quercetin activity was much more than in both extracts. Also, extracts had a weak capacity for Fe^{2+} chelating activity. The MIC values for *C. draba* aerial and underground part extracts were 100 mg/ml, while their MBC values were 150 mg/ml. The aerial part extract exhibited higher ZOI values. Our findings suggest that this plant can be studied further for use as a natural antioxidant and anti-microbial agent in the pharmaceutical industry.

INTRODUCTION: Free radicals play an essential role in many human disease conditions. Different biochemical reactions in the body generate reactive oxygen species [1]. Suppose cell segments do not appropriately scavenge them. In that case, they cause more than one hundred illnesses: atherosclerosis, ischemia, arthritis, gastritis, reperfusion damage of many tissues, central nervous system damage and malignant growth [2, 3]. Because of environmental pollution, poisons, radiation, chemicals, deep-fried and spicy foods and physical stress, free radicals cause the

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decrease of immune system antioxidants, change in gene expression and induce unusual proteins [4, 5]. Antioxidant-rich foods play an essential role in the prevention of cancers, cardiovascular and neurodegenerative diseases [6-8]. Today there has been an interest in the therapeutic potentials of medicinal plants as antioxidants in diminishing such as free radical-induced tissue injury [9-11]. Natural medicines, especially therapeutic plants, serve as alternatives for and the basis of modern medicines, particularly those against infectious diseases [12]. Since, synthetic antioxidants are poisonous at higher concentrations [13-15] medicinal plants for natural antioxidants have raised over the years [16]. Herbal therapy is one appropriate way to treat infectious diseases caused by drug-resistant bacteria [17-20]. Thus, the use of plant extracts, which contain anti-microbial properties including *Cardaria draba* (L.) Desv. may be effective for the treatment of bacterial infections [21-24]. *C. draba* usually known as hoary cress or white top is a permanent herb that reproduces by seeds and horizontal creeping roots [25]. The stem is fairly stout, spreading or erect, branched, 10 to 80 cm tall, covered sparsely to heavily with soft hairs [26] **Fig. 1.**

FIG. 1: *CARDARIA DRABA*

C. draba is local to western Asia, including in most areas of Iran and eastern Europe, also this plant is an invasive species in North America, presented by contaminated seeds in the early 1900s [25]. It usually grows in a broad range of disturbed habitats including rangeland, cultivated land, pastures, waste areas, along roadsides and is known to thrive in irrigated or riparian areas 27 especially. Also, the leaves and seeds of this plant have expectorant and purgative properties [28]. Generally, studies have demonstrated that *C. draba* can be utilized for the right management of numerous illnesses [25-27]. The present paper aimed to determine antioxidant and anti-microbial potential and HPLC analysis of phenolic compounds in the *C. draba* extract to provide a broad spectrum property from this plant.

MATERIALS AND METHODS: Trichloroacetic acid (TCA), ascorbic acid, EDTA, ferric chloride, butylated hydroxyl anisole (BHA), gallic acid, quercetin, potassium ferricyanide, sulfanilamide, ferrozine, dimethyl solphoxamide (DMSO), Muller-Hinton agar, 1,1-diphenyl-2-picryl hydrazyl (DPPH), nutrient broth and N-(1- naphthyl) ethylenediamine dihydrochloride, methanol (MeOH), glacial acetic acid bought from Sigma Chemicals Co. (USA) and other materials were analytical grade.

Methods:

Plant Material and Extraction: Aerial and underground parts of the *C. draba* were collected from Geleh Kola Sofla_Kordkheil village near the Sari city in Mazandaran province, Iran. A voucher specimen (No 93-36) is deposited and identified in the Herbarium of the Department of Botany Shahid Beheshti University in Tehran. Aerial and underground parts of the plant were dried in the shade at room temperature and crushed before extraction. A specific measure of each piece of the plant was extracted at 25 °C by the Soxhlet method utilizing methanol. The extract was concentrated by a rotary evaporator and afterward freeze-dried until the solvent is completely eliminated and acquired a solid extract.

Total Phenols Measurement: Phenolic substances were measured by Folin Ciocalteu reagent [29]. A solution of *C. draba* extract (0.5 ml of1:10 g ml-1) or gallic acid as a standard phenolic compound was merged with Folin Ciocalteu and the aqueous solution of sodium carbonate (4 ml, 1 M). The acquired mix was held at 25 °C for 15 min, and afterward, the phenols were measured by colorimetric technique at 765 nm. Concentrations of 50 to 250 were prepared from gallic acid in methanol: water (50:50, v/v), and then a calibration curve was drawn. Total phenol contents are presented based on gallic acid tantamount (mg g^{-1} of waterless mass)**.**

Total Flavonoids Measurement: The colorimetric aluminum chloride technique is utilized to measure flavonoid [30]. *C. draba* extract was merged with 1.5 ml of methanol, 0.1 ml of aluminum chloride 10%, 0.1 ml of potassium acetate 1 M, and 2.8 ml of distilled water. The extract was held at 25 °C for 30 min; a double beam Perkin Elmer UV/Visible spectrophotometer (USA) was used to read the absorbance of the resulting mixture at 415 nm. The calibration curve was obtained by quercetin solutions in methanol at 12.5 to 100 mg ml^{-1}.

DPPH Radical-Scavenging Activity: The 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) is utilized to evaluate the radical scavenging activity of the extracts [10]. Each extract contains various concentrations in the same volume to 100 mM methanolic solutions of DPPH. After 15 min of storage at room temperature, absorbance was read at 517 nm **Fig. 2**. The test was replicated three times. As controls, ascorbic acid, BHA, and quercetin were utilized. The concentration of sample that inhibits 50% of the DPPH free radicals is called the $IC_{50,}$ which is acquired from the dose-inhibition curve. Its axes are the concentration and percentage of inhibition [31].

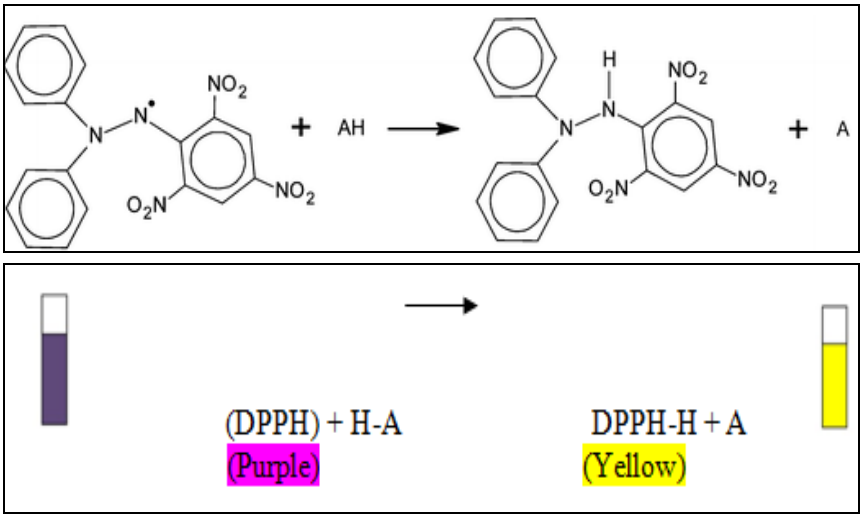


FIG. 2. SCHEMATIC OF THE REACTION BETWEEN DPPH (FREE RADICAL) AND ANTIOXIDANTS [32]

Reducing Power Measurement: The reduction of Fe (III) is commonly utilized to determine electron-giving activity, which is a significant function in the antioxidant effect of phenols. Various concentrations of extracts (25 to 800 µg ml^{-1}) in water were combined with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The resulting mixture was incubated for 20 min a 50 ° C. 2.5 ml of trichloroacetic acid (10%) was eked to the admixture to cease the reaction and afterward centrifuged at 3000 rpm for 10 min. 2.5 ml of the upper portion of the admixture was combined with 2.5 ml of deionized water and 0.5 ml of $FeCl_3$ (0.1%). Absorption was read at 700 nm. Incrementing the absorption of the reaction admixture shows an increase in reducing power. As a positive control, vitamin C was used.

Evaluation of Nitric Oxide-Scavenging Activity: Nitric Oxide (NO) is produced from sodium nitroprusside and is determined by the Greiss reaction as described previously [33]. Sodium nitroprusside at physiologic pH in an aqueous solution produces NO it combines with oxygen and creates nitrite ions, which is evaluated by a Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthyl ethylenediamine dihydrochloride).

The competition of oxygen with NO leads to a reduction in the production of nitrite ions. For the assay, 10 mM sodium nitroprusside in phosphate-buffered saline was merged with various concentrations of each aqueous extract and afterward incubated for 150 min at 25 °C. After incubation, 0.5 ml of Greiss reagent was added. The absorbance of generated chromophore was measured at 546 nm. As a positive control, quercetin was utilized [34].

Metal Chelating Activity: Usually, foods are contaminated by transition metal ions during processing. Bivalent transition metal ions catalyze

oxidative processes and produce free radicals [35]. The extract was eked at a concentration of 50- 1600 µg ml-1to a solution of 2 mM $FeCl_2$ (0.05 ml). 5 mM ferrozine (0.2 ml) is added to initiate the reaction. Subsequent waging and keeping it at 25 °C for 10 min, the absorbance was read at 562 nm. % inhibition of the ferrozine-Fe2+ complex is acquired from the following formula, in which A0 and As are the absorption of the control and absorption of the extract to the standard, respectively. As a positive control, Na2EDTA was utilized.

$$\% \text{ inhibition} = (A0 - As) / As \times 100$$

HPLC Analysis: The phenolic compounds present in the *C. draba* methanolic extracts were analyzed by Lin et al. with partial alterations [36]. The HPLC system consisted of a model K- 1001 pump with an injector (20 µL loop), and a UV-vis detector model K-2600 (Knauer, Germany) set at 290 nm. The analysis was performed using a C18 column (250 mm x 4.6 mm, ODS−35 µm, Perfectsil Target). All solvents were filtered and degassed before entering the column. A gradient solvent system consisting of solvent A (MeOH) and solvent B (H_2O with 9% glacial acetic acid) (conditions: 5% A from 0 to 3 min and kept at 10% A from 3 to 15 min, kept at 30% A from 15 to 27 min and kept at 80% A from 27 to 45 min) were used for separation. The flow rate was set at 0.8 mL min^{-1} and all the measurements were done at room temperature.

Evaluation of Anti-microbial Effect: Anti-microbial efficacy of methanolic extract of the microbial suspension was cultured on the agar Mueller-Hinton media, and various concentrations of extract were eked to wells. Plates were incubated for 24 hours in 37 °C incubators, and the diameter of the zone of inhibition (ZOI) was recorded.

For the tube dilution test various concentrations of the extract were eked to the bacterial suspension and after 24 hours of incubation at 37 °C, the minimum inhibitory concentration (MIC) of the extract was evaluated as the minimum concentration of extract, which inhibited the development of bacteria in tubes. By culturing the content of plates without apparent growth on solid agar media for 24 hours at 37 °C, the minimum bactericidal concentration (MBC) was considered the minimum concentration of extract that inhibited the growth of bacteria on the surface of solid media [37]. Both disk diffusion and tube dilution methods were repeated 3 times for aerial and underground parts of *C. draba*. Chloramphenicol (Loghman Pharmaceutical Co) was used as positive control and %DMSO was used as negative control for tube dilution. Both disk diffusion and tube dilution methods repeated 3 times for aerial and underground parts of *C. draba.*

Statistical Analysis: All tests were replicated three times, and the outcomes were reported as means ± SD. Data were analyzed using analysis of variance (ANOVA) and a P value less than 0.05 was considered statistically significant. The IC50 values were obtained using the linear regression formula.

RESULTS:

Total Phenols Contents: Total phenols contents are stated as gallic acid equivalents which is acquired by calibration curve ($y = 0.0063x$, $r^2 = 0.987$). The total phenolic compound of the extract was 280.65 ± 2.1 (for the aerial part) and 147.58 ± 1.3 (for underground part) mg gallic acid equivalent per gram of solid extract.

Total Flavonoids Contents: The whole flavonoid content of the extracts, equivalent to quercetin (the standard curve equation: $y = 0.0067x + 0.0132$, $r^2 = 0.999$) were 80.8 ±1.2 (for aerial part) and 26.7 ± 0.98 (for underground part) mg g^{-1} in the solid extract.

DPPH Radical-Scavenging Activity: It was found the radical-scavenging activity of extracts increases with increasing their concentrations. IC_{50} of DPPH Radical-Scavenging Activity for the aerial part was 403 ± 2.3 µg ml^{-1} and for the underground part was 555 ± 3.1 µg ml^{-1}, whereas IC_{50} of standard materials, ascorbic acid, quercetin, and BHA were 5.05 ± 0.12, 5.2 ± 0.43 and 53.9 ± 2.1 µg ml^{-1}, respectively.

Reducing Power: In this method, the presence of reducing or oxidants agents in the sample results in the reduction of Fe^{3+} to Fe^{2+} by electron donation. Then, the amount of Fe^{2+} complex production is determined by creating Perl's Prussian blue at 700 nm. **Fig. 3** is related to the reducing power of the extracts of the *C. draba*. The increase in absorbance at 700 nm indicates improved reduction ability. Increasing the concentration of the extracts

increases their reducing power, but all extracts had a weak reduction ability at a concentration of 25 to 800 µg ml^{-1}. Two types of extracts had no significant difference in the reducing power ($p > 0.05$) but compared to vitamin C as a control, this difference was significant and vitamin C had a higher reducing power ($p < 0.01$).

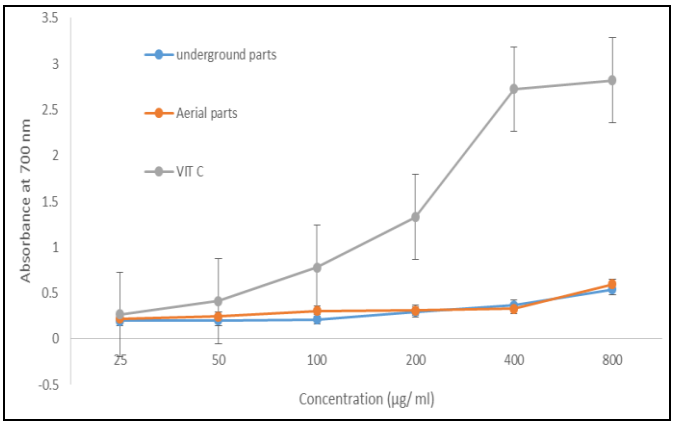
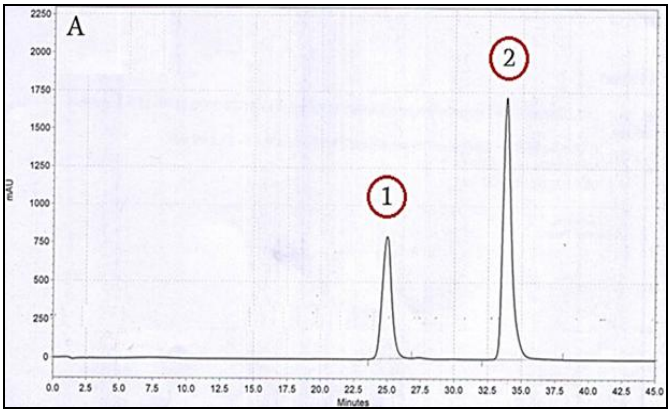


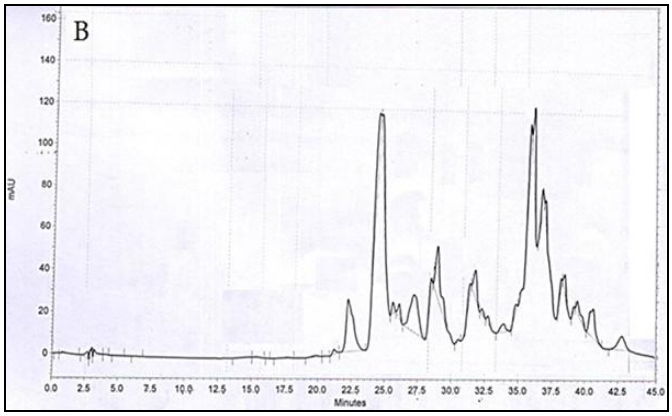
FIG. 3: REDUCING POWER OF *C. DRABA* EXTRACTS AND VITAMIN C, ABSORBANCE INCREASES WITH INCREASING CONCENTRATION

Evaluation of Nitric Oxide-Scavenging Activity: The extract of the aerial part of the *C. draba* had an IC_{50} = 1.18 mg ml^{-1} and a concentration of 1.6 mg ml^{-1} had a percent inhibition of 50.93%, while the IC 50 of the underground part extract of this plant was 0.80 mg ml^{-1} and showed percent inhibition of 57.63 at a concentration of 1.6 mg ml^{-1}. In both extracts, the percentage of inhibition increased with increasing concentrations, although quercetin activity was much more than in extracts (IC_{50} = 37.9 µg ml^{-1}).

Fe^{2+} Chelating Activity: *C. draba* extracts had a weak capacity for Fe^{2+} binding and their IC_{50} for Fe^{2+} chelating ability were 1.38 mg ml^{-1} (for aerial part) and 1.54 mg ml^{-1} (for the underground part). While the EDTA has a strong chelating activity (IC_{50} = 17.34 µg ml^{-1}).

HPLC Separation of Phenolic Acids: The HPLC method using a C18 column and a two gradient solvent system ($MeOH/H_2O$ with 9% glacial acetic acid, flow rate = 0.8 mL min^{-1}) could simultaneously separate 2 phenolic acids within 40 min **Fig. 4**. Methanol extract contained mainly chlorogenic acid and p-coumaric acid.





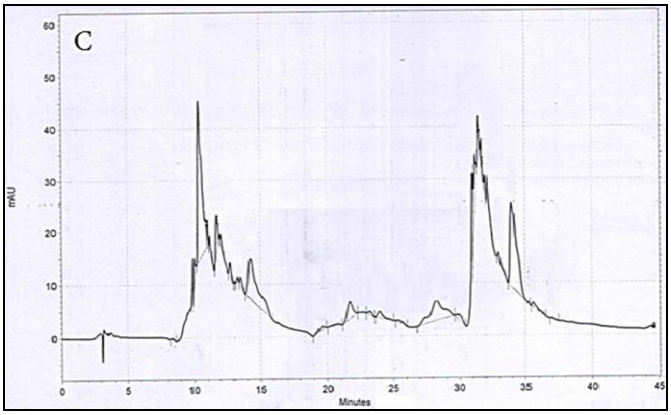


FIG. 4: HPLC PROFILES OF *C. DRABA* EXTRACTS ANALYZED AT 290 NM. A) CHLOROGENIC ACID STANDARD (1) AND P-COUMARIC ACID (2), B) METHANOL *C. DRABA* AERIAL PART EXTRACT AND C) METHANOL *C. DRABA* UNDERGROUND PART EXTRACT

Evaluation of Anti-microbial Activity: The MIC value of *C. draba* aerial part extract against *E. coli* and Klebsiella was 100 mg/ml while its MBC value against both *E. coli* and Klebsiella was 150 mg/ml. The MIC value of *C. draba* underground part extract against *E. coli* and Klebsiella was 100 mg/ml and MBC value against both *E. coli* and Klebsiella was 150 mg/ml. The diameter of the Zone of Inhibition (ZOI) of *C. draba* aerial and underground parts extract are summarized in **Tables 1** and **2.**

TABLE 1: DIAMETER OF ZOI FOR *C. DRABA* AERIAL PART EXTRACT AGAINST *E. COLI* AND KLEBSIELLA IN DIFFERENT CONCENTRATIONS

Concentration of extract (µg/ml)	25	37.5	50	75	100	150
ZOI For *E. coli*(mm)	1.66 ± 0.32	2.33 ± 0.45	2.66 ± 0.65	3.33 ± 0.95	4 ± 1.12	6 ± 1.73
ZOI For *Kalebsiell* (mm)	1.66 ± 0.32	2.33 ± 0.45	2.66 ± 0.65	3.33 ± 0.95	4 ± 1.15	4.33 ±1.24

TABLE 2: DIAMETER OF ZOI FOR *C. DRABA* UNDERGROUND PART EXTRACT AGAINST *E. COLI* AND KLEBSIELLA IN DIFFERENT CONCENTRATIONS

Concentration of extract (µg/ml)	25	37.5	50	75	100	150
ZOI For *E. coli*(mm)	0 ± 0.15	0 ± 0.15	1 ± 0.28	1.66 ± 0.47	2.33 ± 0.66	2.66 ± 0.76
ZOI For *Kalebsiell* (mm)	0 ± 0.15	0 ± 0.15	0.66 ± 0.18	1.33 ± 0.38	2 ± 0.57	2.33 ± 0.66

DISCUSSION: *C. draba* contains the appropriate phenol and flavonoid contents. Phenolic compounds are a type of antioxidant that is known to finisher the activity of free radicals [38]. In general, phenolic and polyphenolic compounds, for example flavonoids, are abundantly found in plant-derived foods and can cause their antioxidant effect [39,] which has a significant effect on human health. The mechanism of action of flavonoids is scavenging or chelating [3, 40]. Therefore, phenolic and polyphenolic compounds may cause antioxidant activity of this plant. As stated earlier, in both types of extracts, DPPH radical-scavenging activity and the percent inhibition of NO increase with increasing their concentrations. Reactive oxygen species and NO is effective in pathological conditions, cancer and inflammation; as a result, scavenging of nitric oxide can be useful, and preventing NO accumulation is an effective therapeutic strategy to combat pathogenic damage to DNA [41, 42].

Also, to measure the capacity of antioxidant effect in the various samples, scavenging the stable DPPH radicals is a simple, fast and sensitive method [43, 44]. In the Fe^{2+} Chelating test, the presence of other chelating agents reduced the formation of the ferrozine complex; thus, the red color decreased. Absorption of Fe^{2+}- ferrozine complex decreased as a dose-dependent manner, that's mean, by increasing the concentration from 0.05 to 1.6 mg ml^{-1}, the activity of the extract increased. It has been made clear that chelating agents by reducing the potential of redox, can stabilize the oxidized forms of metal ions [45]. As shown, the aerial part extract displayed a higher ZOI value, which is proportionate with the aerial part extract's higher phenolic and flavonoid content. The HPLC method separated chlorogenic acid and p-coumaric acid as phenolic compounds in the plant extracts. The anti-microbial effects of chlorogenic acid have been proven in several studies [46-48]. This compound causes the death of bacterial cells by increasing plasma membrane permeability, reducing its protective properties, leakage of nucleotides, and excretion of cytoplasmic macromolecules [49]. The relationship between phenolic and flavonoid compounds of plant extracts is demonstrated in several studies [50]. For instance, a positive relationship between the total phenolic content of grape extract and anti-microbial activity was reported by Baydar *et al.* [51].

Also, it was reported by Mahboubi *et al.* that the anti-microbial effect of Golnar flower extract was decidedly related to the total phenolic and flavonoid content of the extracts [52]. Flavonoids are found in seeds and flowers, and are known as promising anti-microbial agents that can be useful in the treatment of resistant pathogens. Flavonoids' anti-microbial mechanisms are thought to be the inhibition of nucleic acid synthesis, inhibition function of the cytoplasmic membrane, and inhibition of energy metabolism [53].

CONCLUSION: According to the results of this study, it was found that both extracts of aerial and underground parts of *C. draba* had significant

amounts of phenols and flavonoids also, both extracts showed antioxidant and anti-microbial properties in different tests. These characteristics may be due to this plant's high levels of phenols and flavonoids. Depending on the phenol and flavonoid contents, the anti-microbial properties are enhanced. It is recommended to carry out in vivo tests and appropriate dose estimation to evaluate the potential use of the extract of *C. draba* as a natural antioxidant and an anti-microbial agent in the pharmaceutics, as well as the potential for the toxicity and safety of the plant for human, should be studied.

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