Phytochemicals Analysis in Watercress (*Nasturtium Officinale*) Plant Extracts

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**Abstract.** *Nasturtium officinale* is a wild edible species of *Nasturtium* genus that has a great concern of medicinal plant specialist. Traditionally, it was offering rural and local people food security and medicines. Nowadays, these plants become more attractive and interested due to their polyphenol level and antioxidant activities. Therefore, this study aimed to investigate some phytochemicals of aerial portion of this plant including (reducing sugar, carbohydrates, flavonoids, saponin, glycosides, steroids and phenolic compounds) and the effect of altitudes on the total content of phenolic compound and flavonoids. The results showed that *N. Officinale* in all three villages were enriched with steroids, reducing sugar, phenolic compound, glycosides and flavonoids. However, the study was recorded the absence of existing saponin in the three places. Furthermore, the study result highlighted that the high content of phenolic compound (gallic acid = 0.60 mg 100g⁻¹) and flavonoids (quercen = 5.39 mg 100g⁻¹) were achieved from the high altitude compared to the low altitudes phenolic compound (gallic acid = 0.39 mg 100g⁻¹) and flavonoids compound (quercen = 2.93 mg 100g⁻¹). Thus, the evidence of this study shown that watercress can enhance natural medicines for human being.

1. **Introduction**

The relationship and interaction between human and plant traditionally are the base of nature conservation and understanding of the human being [21, 18]. The medicinal plants has a great value for discovering drugs which is known to the human being well and the people are using them for different purposes from the very beginning of history of human [9]. The cures from the traditional folk that came from plants had become a guide for scientists to conduct research for searching a new medicine to promote and maintain health for animals and human [1]. *Nasturtium officinale* is a hardy perennial native to the Europe and Asia belongs to the *Brassicaceae* family (also called *Cruciferae*) that can be grow around and in a water. Watercress is native to India, Africa, Europe and Western Asia [33], however the plant nowadays is distributing globally [15]. This edible plant is found in cold, shallow, gently moving, damp soil, fresh water in lakes, streams, reservoirs, and rivers [33]. It could often find in areas of moving water alongside to springs, on wet soil, riverbanks or flowing streams [7].

Watercress can be commercialised mostly in fresh and in culinary it in soups, salad and some other recipes, the plant considered as a great functional tradition food for the cancer and related diseases prevention [7]. Watercress plant consists of polyphenols and some other related substance which is accepted for activities of antioxidant to wipe reactive oxygen and result to protection of oxidative damage [31]. In addition, it consist of a high level of glucosinolates which is the highest concentration comparing to all other vegetables plants, along with high carotenoids concentration for example b-carotene and lutein [28, 24]. Mentioned phytochemicals had related with different anticarcinogenic
and antioxidant features. The members of *Cruciferae* or *Brassicaceae* family for instance broccoli, cauliflower, mustard green, Brussels, sprouts, cabbage including watercress, had reported to consist of high level of phenolic compound [13]. Glucosinolates is a sort of nitrogen- and sulfur- consisting of glycosides, which are hydrolysed by b-thioglucosidase (myrosinase). Therefore, the glucosinolate hydrolysis products are Isothiocyanates (for example indole 3-carbinol and phenyl ethylisothiocyanate), effect on activities of biotransformation enzyme. *N. officinale* consumption may decrease the danger of cancer by possible antioxidant status modulation and DNA damage reduction [14, 10]. Moreover, [4] reported that the watercress consist of mustard oil which has a lot of medical uses, such as anti-bacterial, and it has an effective protection against anti-cancer of oral which is almost caused by smoking, as well as is used in many other treatment of such cases as scurry, bronchitis, anemia, diuretic, diabetes, kidney, eczema, liver, warts, tumours and tuberculosis [26], moreover as an anti-inflammatory [22]. Traditionally, the plant is used in treating many diseases for example to purify blood, stop haemorrhages, eliminate of excess bile, chest pain, lungs, digestion, throat expectorant, facial scars, gallbladder, iron deficiency, stimulant and skin afflictions [19].

In Kurdistan region of Iraq, harvesting wild edible plant has a strong relation to the rural and local people in the nature. However, *Nasturtium officinale* is well known from its nutritional and phytochemical properties that has not been documented or analysed. Hence, this research aim is to investigate some phytochemicals of aerial portion of this plant including (reducing sugar, carbohydrates, flavonoids, saponin, glycosides, steroids and phenolic compounds) and the effect of altitudes on the total content of phenolic compound and flavonoids.

2. Materials and Methods

2.1 Extracts preparation

Samples collection was conducted at April 2018 to August 1st /2018. After collecting the plants from the fields of Mayea, Brifkani and Zawa villages on different elevations 1661 m,1040 m and 400m respectively, the dryness processes were done inside medicinal plants laboratory /Horticulture Department/College of Agricultural Engineering Science /Duhok University.

All the solvents that used were belong to the American Chemical Society reagent grade (Sigma–Aldrich Chemical Company, St. Louis, MO, USA). The ethanol (1000 mL) was used to extract the plant materials at overnight room temperature on an automatic shaker. The material extractions were concentrated under lessened pressure in an evaporator rotary to achieve the extraction of hexane of plant dried powders (2.43 mg, 2. 00 mg and 2.02 mg), receptively.

2.2 Glycosides Test (Benedict reagent)

1.4 Before hydrolysis

Equal volumes of Benedict's reagent (CuSO₄ and NaOH) and extract were mixed. The mixture was left in a 10 minutes bath water. A reddish coloured precipitation appearance reveals the presence of reducing sugars.

2.3 After hydrolysis

Few drops of concentrated hydrochloric acid were added to 5 ml of extract. The mixture was placed in a water bath for 20 min, and then the acidity was neutralized by adding 2M sodium hydroxide. The same volume of Benedict reagent was added to the mixture and heated in a 10min of water bath. The appearance of the reddish precipitate more than its appearance of solvent before hydrolysis demonstrates the presence of glycosides.

2.4 Saponin test

2.4.1. Aqueous mercury chloride

Aqueous mercury chloride (5%) was used as a reagent for saponin test. One milliliter of extract of plant was mixed with 1ml of the reagent. The appearance of a white precipitation expresses the presence of saponin [12].
2.5 Carbohydrate Test (Molish test)
One milliliter of Molish reagent [(1%) α- naphthol. in absolute ethanol] was mixed to 1ml of extract, then few drops of concentrated sulfuric acid were added slowly on wall tube for three locations. The violet ring appearance demonstrates the presence of carbohydrate [12].

2.6. Flavonoid test
2.6.1. Alcoholic potassium hydroxide reagent
One milliliter of alcoholic potassium hydroxide (5N) was added to 1 ml of extract. The appearance of a yellow coloured precipitation reveals the presence of flavonoids.

2.7. Detection of steroid
2.7.1. Salkowski reaction detection
A few crystals of compounds 1 and 2 were dissolved in chloroform and a few drops of concentrated sulphuric acid were added to the solution, both compounds 1 and 2 formed a reddish color in the upper chloroform layer [12] indicating presence of steroids.

2.7.2. Liebermann-Burchard reaction detection
A few crystals of compounds 1 and 2 were dissolved in chloroform and few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. In this case both compounds 1 and 2 turned to violet blue and finally formed a green color which indicates the presence of steroids [12].

2.8. Phenolic compound test
Filter paper was saturated with extract solution, and then few drops of (1%) ferric chloride were added to the saturated filter paper. The filter paper was exposed to ammonia solution vapour. The appearance of blue or green color in the filter paper indicates the presence of phenolic compounds [12].

2.8.1. Total Phenol Content (TPC)
Total phenolic content of black mulberry fruits was measured according to the methods using Folin-Ciocalteu reagent and tannic acid as a standard [29, 16]. Eight grams of fruits were mixed with 30 ml (0.4 %) oxalic acid using an electrical blender and the mixture was assisted with stirring for 10 min. The residues were removed by filtration using filter paper (Whatman No. 40), and 50 ml of D.W were added to the filtrate to obtain the final concentration of fruits containing 100 mg ml. For each extract and fruits solution (0.1 ml) containing 100 mg extract were taken in a volumetric flask, 46 ml of distilled water and 1 ml Folin-Ciocalteu reagent was added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% sodium carbonate were added and the mixture was allowed to stand for 2 hrs. with intermittent shaking. The blank consisted of 0.1 ml of D.W instead of the extract. Absorbance was measured at 760 nm. All samples were assayed twice. The same procedure was applied to all standard tannic acid solutions (0–2500 mg/50ml), and standard curve was obtained. Results were expressed as milligrams’ of tannic acid equivalent per gram (mg TAE g) of extract.

2.8.2. Total Flavonoid Content (TFC) determination
The method that described by [6] was used to total flavonoid content determination. Half milliliter of each extract (0.1 gm/ml) in methanol was separately mixed with 1.5 ml of methanol, and 0.1 ml of 10% aluminium chloride, then 0.1 ml of potassium acetate and 2.8 ml of distilled water were added. Then the mixture remained 30 minutes at room standard temperature. The reaction mixture absorbance was measured at 415 nm with UV/visible spectrophotometer. All samples were assayed in duplicate. The same procedure was applied to all standard quercetin solutions (12.5–100 μg/ml) in methanol and a standard curve was obtained. Results were expressed as milligrams of quercetin equivalent per gram (mg QE g) of extract.
3. Results and Discussion:

3.1. Phytochemical analysis

Phytochemical screening of the crude was shown in table (1) and figure (1). Results revealed the presence of medicinally active constituents. Seven phytochemical characters of *Nasturtium officinale* (watercress) were investigated. They are summarized in table (1) and figure (1) including carbohydrates, reducing sugar, glycosides, flavonoids, steroids, saponin and phenolic compounds. These were present in all the studied plant extracts including the various three areas especially the plants collected from KM. village showed much more quantity of these compounds compared to the others. Saponin was absent in the three extracts.

The phytochemical screening and quantities estimation of the aerial portion of plant study showed that the plant rich in carbohydrates, reducing sugar, glycosides, flavonoids, steroids and phenolic compounds. They were known to show medicinal activity as well as exhibiting physiological activity [8]. These results in agreement with [20, 2]. The plants studied here can be seen as potential source of useful drugs. Further works are going on this field in order to isolate, purified, identified, characterize and introduce the structure of bioactive compounds.

| Table (1) Phytochemical analysis of *Nasturtium officinale* extracts: Mayea, Brifkani, Zawa extracts. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| extract Reagent                               | Mayea ext.     | Brifkani ext.   | Zawa ext.       | Observations    |
| Mercury chloride                              | -              | -               | -               | Absence of saponin |
| Molish test                                   | ++             | +               | +               | Presence of carbohydrates. |
| Benedict reagent (before hydrolysis)          | +              | +               | +               | Presence of reducing sugar. |
| Benedict reagent (after hydrolysis)           | +              | +               | +               | Presence of glycosides. |
| (5N)Alcoholic KOH                             | ++             | +               | +               | Presence of flavonoids. |
| Liebermann-Burchard Sulphuric acids           | ++             | +               | +               | Presence of steroids. |
| Salkowski reagent Sulphuric acids             | ++             | +               | +               | Presence of steroids. |
| FeCl3+ ammonia vapor                          | ++             | +               | +               | Presence of phenolic compound |
Fig. (1) Phytochemical screening of using different reagent. A. Molish test appearance the violet ring indicated to presence of carbohydrate, Mayea .ext. on the right, B. Benedicat reagent appearance the radish precipitate indicated to presence of reducing sugar Mayea ext., C .Alcoholic (KOH ) reagent appearance the yellow color indicated to presence of flavonoids Mayea. ext., D.FeCl₃ reagent appearance the yellow or green color indicated the presence of phenols Mayea. ext. E. Libermann test appearance the violet blue and formed green color indicated to presence of steroids. And F. Salkowski test appearance radish color upper chloroform layer indicated to presence of steroids.

3.1.1. Total Phenols
3.1.1.1. Total polyphones (TPC)

Results of this research showed that the phenolic compounds levels were greatly affected by altitudinal levels of variation. Figure (2) clarifies that the high level of phenolic compounds was obtained from Mayea area (0.60g gallic acid eq.100g⁻¹), this was the highest location among this research with the altitude 1661 m. Beside the lowest level of phenoilc compounds was found in Zawa village (0.39mg gallic acid eq.100g⁻¹), the area was low with the altitude 450 m. These results indicated the influence of the elevation on the total content of phenolic compounds. Phenolic compounds, also called polyphones are metabolic products distributed in plant foods. These compounds had many biological and pharmacological characteristic that could provide protection against chronic disease. They have antioxidant activity more than vitamins. They are able to neutralize the free radicals oxidative [30, 27].
3.2. Total Flavonoids Content (TFC)

Mayea village plant materials extract showing higher contain of flavonoids compounds, more than other Brifka and Zawa the values were (5.39, 3.29 and 2.93 mg quercen eq.100g⁻¹) respectively for all three different areas, Mayea has a high altitudes comparing to the Brifka and Zawa have a low altitudes. Flavonoids are one class of secondary plant metabolites that are known as vitamins. These metabolites are responsible on yellow and other pigments which play a remarkable role in the colours of plants. Flavonoids are easily digested by human.
Results were agreed with the result that approved by [20] that the contents of secondary metabolites for instance total flavonoids and phenolic were high in the aerial parts of *Nasturtium officinale* that were collected from high altitude areas of Touskachmesh (1400 m) in comparison with the lowest amounts of these compounds from the plant material that were collected from low altitude (200 m) of Nosrat Abad.

These results are also in agreement with those of researchers, whom determined the total phenols and total flavonoids content in *Nasturtium officinale* plant like, [20] whom determined the phytochemical compounds of aerial parts of watercress *Nasturtium officinale*, the results showed that the total phenols content and total flavonoids content were obtained. [34] Investigated the total phenolic content of *Nasturtium officinale* extract and total flavonoids content. [2] determined the total phenolic compounds and total flavonoid compounds in methanol extract of three parts of *Nasturtium officinale* plants including flowers, leaves and stems.

Other studies had also reported the influence of mineral nutrients, drought, light intensity and altitude on plants growth and essential oil contents [17, 3]. [5] also confirmed that the differences in the chemical composition are affected by various altitudes due to the environmental factors such as altitude and geographical position. The researchers concluded that the ecological factors of habitat such as altitude and physiochemical properties of soil [32, 25] not only can effect on plant vegetative growth, can also change the quality and quantity of essential oils and chemical compounds in aromatic and medicinal plants.

As it is known in upland, the humidity is less and has more sunshine rays especially ultra-violate rays which are powerful to make the plant dense. Physiological fluids as essential chemical composition of *Phlomis cancellata* have followed this version. A number of researchers have reported that altitude increasing levels can decrease the amount of the chemical compounds of essence [23, 11]. Environmental conditions are changes due to altitude and latitude; as known the plants in upland are expose to hard environmental conditions more than the plants that grow in down land which affect its growth and development. Development period usually is characterized by low temperature, less humidity and more sunshine rays, especially UV rays. So more morphological, anatomical and
physiological changes will happened to resist the environmental stress; for instance, vegetative and root system [28].

4. Conclusion
The study investigated some phytochemicals of aerial portion of this plant including (reducing sugar, carbohydrates, flavonoids, saponin, glycosides, steroids and phenolic compounds) and the effect of altitudes on the total content of phenolic compound and flavonoids. The finding of this analysis showed that the watercress in all three places were rich by the phenolic compound, reducing sugar, steroids, flavonoids, glycosides in all three different villages, the highest village had a highest contents of all these compound which was Mayea village. While the study was revealed the absence of existing saponin in the three extracts. Moreover, the study result recorded that the high content of phenolic compound and flavonoids were achieved from the high altitude compared to the low altitudes.

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