Defence Life Science Journal, Vol. 6, No. 4, October 2021, pp. 298-304, DOI : 10.14429/dlsj.6.16281


# A Comparative Study of Antioxidant Potential and Phytochemical Contents of different Extracts of Wild *Nasturtium Officinale* W.T. Aiton Collected from Kumaun Region of Uttarakhand

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**ABSTRACT**

*Nasturtium officinale* W. T Aiton or "watercress" (Brassicaceae) is a nutritionally valuable plant that is the richest source of carotenoids, polyphenols, iron, calcium, iodine, folic acid, essential vitamins and minerals. It is traditionally used for their appetiser, anti-scorbutic, stimulant, diuretic and detoxifying properties. The present study was conducted to investigate the antioxidant potential, phytochemical contents (total phenolic, flavonoids and tannin contents) as well as a preliminary phytochemical screening of different extract of aerial parts of *Nasturtium officinale* collected from the Kumaun region. The different extracts showed significant antioxidant activity as well as total phenolic, flavonoids and tannin contents. The preliminary phytochemical screening showed the presence of several phytochemical constituents such as carbohydrates, proteins, amino acids, glycosides, alkaloids and others. The hydroalcoholic extract possess significantly ($P<0.05$) higher antioxidant potential with $IC_{50}$ value (0.333 mg/ml, 0.509mg/ml)and $EC_{50}$ value (3.537 mg/ml) by 2,2-diphenyl-1-picrylhydrazyl(DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)(ABTS), and ferric-reducing antioxidant power (FRAP) methods, respectively, as well as highest total tannin, flavonoid and phenolic contents among all the extracts. These results provide substantial evidence that the plant *Nasturtium officinale*has antioxidant potential and valuable sources of phytochemicals.

**Keywords:** Total phenolic contents; Nutritionally valuable; Preliminary phytochemical screening; Hydroalcohlic extract; $IC_{50}$ value

## 1. INTRODUCTION

Medicinal plants have been used by humans to relieve and cure many diseases from ancient times. In many parts of the world, traditional medicine replaces conventional medicine. Many medicinal plants exhibit antioxidant activity, which is attracting considerable attention among research teams for its involvement in the treatment and prevention of diseases such as cardiovascular diseases, cancer, diabetes, hypertension, atherosclerosis and Alzheimer's disease[1]. Antioxidants are desired to prevent the formation of reactive oxygen and nitrogen species and inhibit oxidative stress in cells, which are generated *in-vivo*. The endogenous antioxidant defences system of the body like superoxide dismutases, metal-binding proteins and $H_2O_2$-removing enzymes are insufficient to completely avoid damage[2]. Plants-based natural products as well as their derivatives are well known for their pharmaceutical importance due to their bioactive compounds. Several medicinal plant extracts have been recognised for antioxidant activities[3]. The reactive oxygen species (ROS) are highly reactive and transient, which causes destruction and irreversible damage to the cellular constituent's *viz.* proteins, lipids, DNA and lipoproteins. These events can cause tissue damage and cell death, which can lead to a variety of human diseases such inflammation, arthritis, cancer, diabetes, atherosclerosis, and cirrhosis and emphysema. Cells possess several antioxidant defence mechanisms including anti-oxidative enzymes and small molecules such as glutathione and vitamins C and E, which helps prevent reactive oxygen species destructive effects[4].

*Nasturtium officinale* W. T Aitonor, commonly called as watercress, member of the Brassicaceae family and is grown as a nutritious, perennial, and flavorful food that is popular in many countries. It is a richest source of phytochemicals similar to other members of the family[5]. *N. officinale* and *N. microphyllum* are the two common species found in the India, and distributed in the Madhya Pradesh, Tamil Nadu, Goa, Sikkim, Punjaband Uttarakhand. The latter has only been documented in Himachal Pradesh and Uttarakhand[6]. In the Kumaun region of Uttarakhand it iscommonly known as 'Piriya', 'halim'[7]. It also contains glucosinolates (mustard oil glucosides)[6].

*N. officinale* is augmented with carotenoids, polyphenols and α-tocopherol. It is a major origin of iron, calcium, iodine, folic acid, tannins, flavonoids, terpanoids and many other glycosides. It contains essential vitamins and minerals like lutein and zeaxanthin[8].

It is a valuable source of vitamin A, B, $B_2$ and C and it is a good detoxifying herb. It's high content of vitamin C and mineral make it's a remedy that is particularly valuable for

Received : 07 September 2020, Revised : 30 May 2021
Accepted : 12 August 2021, Online published : 06 October 2021

chronic illness. This plant is thought to stimulate the appetite and to relieve indigestion to help in case of chronic bronchitis to be generally stimulating and to act as a powerful diuretic[9]. It is also consumed as a raw salad and as a vegetable and acts as an appetiser, anti-scorbutic, stimulant. Throughout India it is cultivated and wildly found in Punjab and hill areas[7]. Elfalleh *et al.* (2019)[10] carried out a comparative study in the different extracts of *Stachys tmoela* plant. The study concluded that antioxidant activity tested with different methods revealed no significant differences in the different extract of the aerial part of the plant and also determined the phenolic contents.

The main purpose of this study was to evaluate total phenolic, tannin and flavonoid contents and antioxidant activity in different extracts of aerial parts of *Nasturtium officinale* that has been used by the rural healers in the Kumaun region of Uttarakhand to prevent and treat inflammatory conditions and as nutraceuticals to improve appetite.

**Figure 1. Aerial parts of *Nasturtium officinale* W. T Aiton plant, collected from Uttarakhand**

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

The aerial parts of *N. officinale* were wildly collected from Almora district of Kumaun region (Uttarakhand) (Fig. 1). The collected plant material was air-dried and blended into the powder. The powder of aerial parts was stored in an air-tight container. The dried powder sample (50 gm) was extracted with 250 ml of water, ethanol, and ethanol-water mixture (7:3) by the soxhlet extraction method separately. The samples used for the determination of phytochemical constituents and antioxidant activity was prepared by using 100 mg of aqueous, ethanolic and hydroalcohlic extract in 10 ml in respective solvents. The concentration of the prepared sample is 10 mg/ml.

### 2.2 Chemicals

Sodium carbonate ($Na_2CO_3$), Potassium permagnate ($KMnO_4$), Ferric chloride ($FeCl_3$) Potassium per sulphate ($K_2S_2O_8$), Aluminium trichloride ($AlCl_3$) Potassium acetate ($CH_3COOK$), DPPH (1,1-diphenyl-2-picryl-hydrazyl), Ascorbic acid, Tannic acid, ABTS (2, 2- azino-bis(3-ethyl benzothiozoline-6- sulfonic acid) diammonium salt, Sulphuric acid ($H_2SO_4$), Lead acetate $(CH_3COO)_2Pb$, Oxalic acid and other reagents were purchased from Sigma Chemical Co, Ltd. USA.

### 2.3 Preliminary phytochemical screening

The different phytochemical tests were performed on the aqueous, hydroalcohlic and alcoholic extract of the plant obtained. The different tests were performed to screen the presence of different chemical constituents such as carbohydrates, proteins, amino acids, glycosides, alkaloids, tannins and phenolic compounds, flavonoids[11].

### 2.4 Evaluation of antioxidant potential

#### *2.4.1 DPPH Free Radical Scavenging Assay*

The DPPH method was used for estimating Inhibitory Concentration ($IC_{50}$) of the aqueous, ethanolic and hydroalcohlic extract according to the Kedare *et al.* (2011)[12]. The methanolic solution of DPPH (2 ml of 0.1mmol) was added to the different aliquots (20-100 µl) of each extracts (10 mg/ml) and then the final volume was made up to 3 ml in each test tube and absorbance were observed after 40 min. at 517 nm against blank (methanol). The reference standard used was ascorbic acid[13,14]. The percentage FRSA (free radical scavenging activity) of DPPH radicals were calculated and further $IC_{50}$ value was determined as:

FRSA (%) = [(Ac - At)/ Ac] ×100, Where, Ac = absorbance of control, At = absorbance of test.

The $IC_{50}$ value calculated as:

InhibitoryConcentration($IC_{50}$) value (mg/ml) = (Concentration of test (close to 50% FRSA) / FRSA close to 50 %) × 50

#### *2.4.2 ABTS Free Radical Scavenging Assay*

The total antioxidant activity was estimated as per the Re, R *et al*. (1999)[15] method, the different concentrations (20-100 µl) of the extracts were added into test tubes and then makeup the volume up to 1ml with distilled water. 1 ml of ABTS solution was added. Test tubes were shaken and kept in dark for 5-7 minutes. The absorbance of the all sample solutions were observed at 734 nm against methanol as blank. In the assay ABTS radical cation ($ABTS^{\bullet+}$) was produce, when ABTS reacts with the potassium persulphate. $ABTS^{\bullet+}$ is a blue-green chromogen which show absorbance maxima at 734 nm. The antioxidant activity observed according to the extent of decolorisation. The antioxidants change the coloured radical cation ($ABTS^{\bullet+}$) to colourless ABTS, which is due to its hydrogen donating availability. The percentage FRSA and $IC_{50}$ value were calculated similarly as mentioned above[14].

#### *2.4.3 FRAP Free Radical Scavenging Assay*

The reducing ability of medicinal plants was determined according to the method used by Adele *et al.* (2010)[17] with modifications. Different concentrations of the extracts were added into test tubes and then make up the volume up to 1ml with distilled water. After that 2.5 ml of phosphate buffer (0.2M, pH 6.6) were added to the above solution followed by the 2.5

ml of potassium ferricyanide (1 %). The resultant solution then incubated at $50^0$C for 20 min, following the addition of the2.5 ml of trichloroacetic acid (10 %).The mixture was then centrifuged for 10 min (3000 rpm). Further, taken 2.5 ml of the upper layer of the resultant solution and mixed with 2.5 ml of distilled water following the addition of 0.5 ml of $FeCl_3$ solution (0.1 %). Immediately, after that measured the absorbance at 700 nm. The reference standard used was ascorbic acid[14,16]. The reducing ability was measured in terms of $EC_{50}$ value (mg/ml).

### 2.5 Evaluation of phytochemical constituents

*2.5.1 Total Phenolic contents*

The total phenolic contents (TPC) of the extracts estimated by using the Folin- ciocalteu method[18]. As per the method, 100 µl of the extracts were taken into the text tubes followed by the addition of the distilled water (3 ml). Then 0.5 ml of the folin-ciocalteu reagent was added. Mixed the resultant solution and added 2 ml of 20 per cent sodium carbonates solution just after 3 min. The solutions was mixed thoroughly and boil for atleast 1 min in a water bath. The resultant solution turns to blue colour solution by the complex formation, which formed due to the reaction of sample with the phosphomol**y**bdic acid. The absorbance was measured at 650 nm. The total phenolic contents were measured in terms of catechol and the values expressed as mg catechol equivalent/g (mgCE/g) on a dry weight basis[14,19].

*2.5.2 Flavonoid Contents*

The aluminium chloride method was used to determine the flavonoid contents (TFC) of the extracts[20]. As per the method, 100 µl of the extracts were taken into the text tubes followed by subsequently addition of the 80 per cent and 95 per cent ethanol. Then the aluminium chloride solution (100 µl) was added to each tubes except the blank sample, followed by the addition of 100 µl potassium acetate solution. The solution was then thoroughly mixed in vertex (1500 rpm) and incubated (30 min). The absorbance of the resultant solution was measured at 415 nm. The flavonoids contents were measuredin terms of quercetin and the values expressed as mg quercetin equivalent/g (mg QE/g) on a dry weight basis[14, 21].

*2.5.3 Tannin Contents*

The tannin in plant extract was determined by folin-denis method[22]. As per the method, 100 µl of the extracts were mixed with 30 ml of water in a volumetric flask and 2.5 ml folin-denis reagent was added followed by the addition of 35 per cent sodium carbonate solution. The resultant solution was mixed thoroughly and make-up the volume up to 50 ml with distilled water. Then placed the flask to heating plate for 30 min (10-$20^0$C). The absorbance was observed at 700 nm. The tannin contents were measured in terms of tannic acid and the values expressed as mg tannic acid equivalent/g (mgTAE/g) on a dry weight basis[14].

*2.5.4 Statistical Analysis*

The results were expressed as Mean ± SEM (Standard error mean) (n=3). The results were interpreted by ANOVA (one-way analysis of variance) followed by the Duncan's test ($P<0.05$) by the SPSS 16.0 Software. The difference among the mean value was consider to be significant ($P<0.05$).

**Table 1. Preliminary phytochemical screening of various extracts of aerial parts of *Nasturtium officinale* plant.**

| Organic components | Tests | HAE | AQE | ALE |
|---|---|---|---|---|
| Carbohydrates | Molisch test | Present | Present | Present |
| | Benedicts test | Present | Present | Present |
| | Fehling's test | Present | Present | Present |
| | Iodine test | Present | Present | Present |
| Proteins | Biuret test | Present | Present | Present |
| | Million's test | Present | Present | Present |
| Amino acids | Ninhydrin test | Present | Present | Present |
| | Test for cysteine | Present | Present | Absent |
| Alkaloids | Mayer's test | Absent | Absent | Absent |
| | Hager's test | Present | Absent | Present |
| | Dragendroff's test | Absent | Absent | Present |
| Glycosides | Legal test | Present | Absent | Present |
| | Killer killiani test | Present | Absent | Present |
| | Foam test | Present | Present | Present |
| | Test for anthraquinones | Absent | Absent | Absent |
| Steroids | Salkowski reaction | Absent | Absent | Absent |
| Flavonoids | Shinoda test | Present | Present | Present |
| | Sulphuric acid test | Present | Absent | Present |
| Vitamins | Test for vitamin A | Present | Present | Present |
| | Test for vitamin C | Present | Present | Present |
| Tannins and phenolic compounds | 5%$FeCl_3$ test | Present | Absent | Present |
| | Bromine water test | Present | Present | Present |
| | Dilute $HNO_3$ test | Absent | Present | Absent |
| | Lead actetate test | Present | Absent | Present |
| | Acetic acid test | Present | Present | Absent |

HAE- hydroalcoholic extract; AQE- aqueous extract; ALE-alcoholic extract

## 3. RESULTS

### 3.1 Preliminary Phytochemical Screening

The different phytochemical tests were performed on ethanolic, aqueous and hydroalcohholic extracts of the aerial parts of the plant. The results revealed that the plant extracts showed the presence of several phytochemicals, such as reducing sugars, flavanones, tannins, polyphenols, alkaloids, vitamins, and protein. While, the steroids and anthraquinones were absent in all the extract of aerial parts of *N. officinale*. (Table 1).

### 3.2 Evaluation of Antioxidant Activity

Various assays have been used to determine the antioxidant potential of aerial parts of *N. officinale*. The methods used are ABTS and DPPH free radical scavenging assay, and FRAP free radical scavenging assay, were carried out in replication (n=3). The ascorbic acid (0.1 mg/ml) used as reference standard for the antioxidant assays.

#### *3.2.1 DPPH Free Radical Scavenging Assay*

The antioxidant activity was estimated in terms of $IC_{50}$ (inhibition concentration 50), minimum $IC_{50}$ value represents the maximum antioxidant activity**.** The $IC_{50}$ values of various extract of aerial parts of *Nasturtium officinale* using free radical scavenging activity using DPPH method were found in range between 2.226 to 0.509 mg/ml. The results revealed that the hydroalcohlic extract showed the minimum $IC_{50}$ value (0.509 mg/ml) followed by the aqueous (0.959 mg/ml) and ethanolic extract (2.226 mg/ml). Among the different extracts, the hydroalcohlic extracts displayed better antioxidant activity followed by the other extracts of the plant. The reference standard used in the study is ascorbic acid (0.00967 mg/ml). Figure 2 shows the antioxidant activity in aqueous, ethanolic and hydroalcoholic extracts of *Nasturtium officinale*.

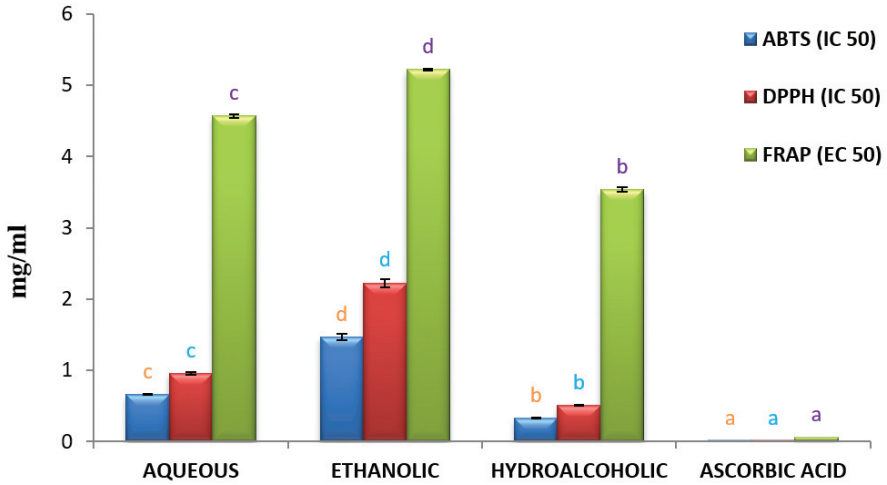


**Figure 2. Free radical scavenging activity (mg/ml equivalent to ascorbic acid on a d.w. basis) of various extracts of aerial parts of *Nasturtium officinale* W. T Aiton plant using ABTS, DPPH and FRAP methods with different letters (a-d) showing significant difference at P < 0.05 probability level (Duncan's test).**

#### *3.2.2 ABTS Free Radical Scavenging Activity Assay*

The antioxidant activity was estimated in terms of $IC_{50}$ (inhibition concentration 50), minimum $IC_{50}$ value represents the maximum antioxidant activity**.** The $IC_{50}$ values of various extract of aerial parts of *Nasturtium officinale* using free radical scavenging activity using ABTS method were found in range between 1.469 to 0.333 mg/ml. The results revealed that the hydroalcohlic extract showed the minimum $IC_{50}$ value (0.333 mg/ml) followed by the aqueous (0.660667 mg/ml) and ethanolic extract (1.469 mg/ml). Among the different extracts, the hydroalcohlic extracts displayed better antioxidant activity followed by the other extracts of the plant. The reference standard used in the study is ascorbic acid (0.00393 mg/ml). Figure 2 shows the antioxidant activity in aqueous, ethanolic and hydroalcoholic extracts of *Nasturtium officinale*.

#### *3.2.3 FRAP Free Radical Scavenging Activity Assay*

The anti-oxidant activity was calculated as effective concentration 50 ($EC_{50}$), lower the $EC_{50}$ value, higher the anti-oxidant potential. The $EC_{50}$ values of various extract of aerial parts of *Nasturtium officinale* using free radical scavenging activity using FRAP method were found in range between 5.22 to 3.537 mg/ml. The results showed that the hydroalcohlic extract of the plant shows highest antioxidant potential with lower $EC_{50}$ value (3.537 mg/ml) followed by the aqueous (4.566667 mg/ml) and ethanolic extract (5.22 mg/ml). Among the different extracts, the hydroalcohlic extracts displayed better antioxidant activity followed by the other extracts of the plant. The $IC_{50}$ value of Ascorbic acid was 0.0574mg/ml. Figure 2 shows the antioxidant activity in aqueous, ethanolic and hydroalcoholic extracts of *Nasturtium officinale*.

### 3.3 Evaluation of phytochemical constituents

#### *3.3.1 Total Phenolic Contents*

The total phenolic content (TPC) was determined using catechol as standard. The TPC was expressed as mg catechol equivalent (CE)/g on a dry weight basis of the extract. The total phenolic contents present in hydroalcohlic, aqueous and ethanolic extracts of aerial parts of *Nasturtium officinale* varied from 0.266 to 4.842 mg CE/g d.w. The findings showed that among three different extracts of the plant, hydroalcohlic extract shows maximum total phenolic content 4.842 mg/g followed by the aqueous (2.287 mg/g) and ethanolic extract (0.266 mg/g). Figure 3 shows the total phenolic contents in aqueous, ethanolic and hydroalcoholic extracts of *Nasturtium officinale*.

#### *3.3.2 Flavonoids Content*

The flavonoid contents (TFC) was determined using quercitin as standard. The TFC was expressed as mg quercitin equivalent (QE) /g on a dry weight basis of the extract. The flavonoidcontents present in hydroalcohlic, aqueous and ethanolic extracts of aerial parts of *Nasturtium officinale* varied from 3.849 to 7.509 mg QE/g d.w. The findings showed that among three different extracts of the plant, hydroalcohlic extract shows maximum flavonoids content 7.509 mg/g.

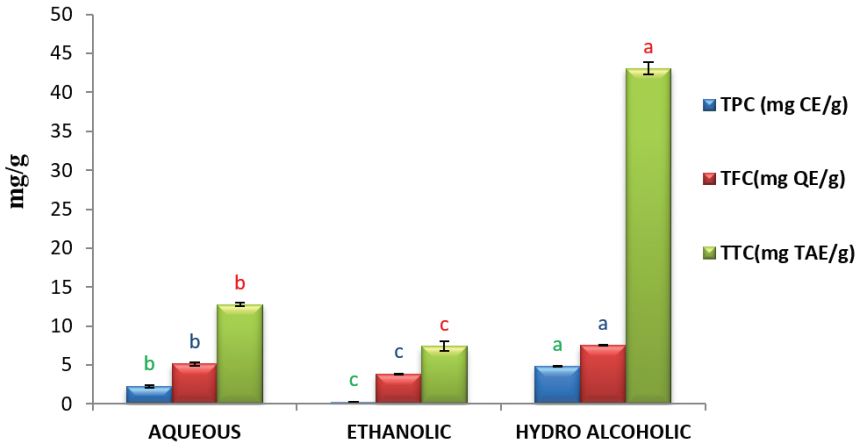


**Figure3. Total phenolic content, total flavonoids content, total tannins content of various extracts of aerial parts of *Nasturtium officinale* W. T Aiton with different letters (a-c) showing significant difference at P < 0.05 probability level (Duncan's test).**

followed by the aqueous (5.136 mg/g) and ethanolic extract (3.849 mg/g). Figure 3 shows the total flavonoids content in aqueous, ethanolic and hydroalcoholic extracts of *Nasturtium officinale*.

### *3.3.3 Tannin Contents*

The tannin content (TTC) was determined using tannic acid as standard. The TTC was expressed as mg tannic acid equivalent (TAE)/g on a dry weight basis of the extract. The tannincontents present in hydroalcohlic, aqueous and ethanolic extracts of aerial parts of *Nasturtium officinale* varied from 7.409 to 43.055 mg TAE/g d.w. The findings showed that among three different extracts, hydroalcohlic extract of aerial parts of plant shows maximum tannins content of 43.055 mg/g followed by the aqueous (12.731 mg/g) and ethanolic extract (7.409 mg/g). Figure 3 shows the total tannin contents in aqueous, ethanolic and hydroalcoholic extracts of *Nasturtium officinale*.

## 4. DISCUSSION

The phytochemicals which are chargeable for antioxidant activity have attained significant reaction lately for its efficient contribution in prevention of human disorder as well as nutraceuticals. The hydrogen donating capability of antioxidants causes scavenging of free-radical. The free-radicals scavenging ability of the plants might be due to the presence of phytochemical components which includes phenolic compounds that are able to converting the free radicals into more stable products[23]. The anti-oxidative biomolecules present in the plants have received increasing interest these days for their capability function in human ailment prevention[24, 4].

The present study deals with the preliminary phytochemical screening, antioxidant potential and phytochemical contents present in the different extracts of aerial parts of *Nasturtium officinale.* The findings revealed the presence of different phytochemicals, antioxidant capability and phytochemical contents of the hydroalcoholic, aqueous and ethanolic extracts of the plant. The recent work by Meriem *et al*. (2017)[25] on ethanolic extract of the leafy-stems of *N. officinale* to determined the antioxidant activity. The findings suggested that the ethanolic extract showed antioxidant potential in concentration dependent manner. The present study, compared the presence of phytochemicals, antioxidant capabilities and phytochemical contents present among the hydroalcoholic, aqueous and ethanolic extracts against various free radicals. The results of present study suggested that thevariation observed among the total phenolic, flavonoids and tannin contents, and the antioxidant potential might be dependent on the choice of extraction solvent. The antioxidant activity was estimated in terms of $IC_{50}$ value, minimum $IC_{50}$ value represents the maximum antioxidant activity; therefore, the hydroalcoholic extract displayed better antioxidant activity followed by the other extracts of the plant. Similarly, the hydroalcoholic extract showed the highest total phenolics, flavonoids and tannin contents among other extracts.

The phenolic compounds present in the sample produced a complex by the reaction of phosphotungestic and phosphomolybdic acids (folin-ciocalteu reagent) and the free radical scavenging activities of the phenolic compounds is due to the metal-ion chelating and hydrogen-donating properties[26-27]. Flavonoids are polyphenolic compounds which act as endogenous antioxidants as a scavenger with various mechanisms. Specifically, flavones are the most powerful flavonoids as antioxidants. Tannins are active compounds of secondary metabolites that are known to have antioxidant activity, are very complex components of organic substances, which include phenolic compounds that are tough to separate, difficult to crystallise[28]. The result of the antioxidant activity of the plant indicates distinctive outcomes on the basis of the nature of the solvent used for extraction and particularly to the numerous methods used for the analysis of various antioxidant phytoconstituents and antioxidant activity[1]. The present study shows that significant tannins, phenolic and flavonid compounds were present in each extract of *Nasturtium officinale.* The hydroalcoholic extract contains the highest concentration of phenolic compounds, flavonoids and tannins among aqueous and ethanolic extract.

## 5. CONCLUSION

The study concluded that among different extracts of aerial parts of *Nasturtium officinale,* the hydroalcoholic extract displayed significantly maximum antioxidant activity followed by the aqueous extract and minimum activity observed in the ethanolic extract. As far as preliminary phytochemical evaluation and phytochemical constituent are concerned, the hydroalcoholic extract exhibited the significant higher total phenolic, tannin and flavonoid contents, and presence of several phytochemicals. Consequently, the findings suggested that the aerial parts can be utilised as a direct source of antioxidants. Therefore, *Nasturtium officinale* can be used as an accessible source of natural antioxidants with consequent health benefits.

Further, there is a need of identification of bioactive components of the plant with their pharmacological effects.

## 6. ACKNOWLEDGEMENT

The authors are grateful to Head, Department of pharmaceutical sciences, Bhimtal forproviding necessary facilities. The authors are also thankful to Dr H. K. Pandey, Scientist 'F', DIBER, DRDO, Pithoragarh for his guidance and support.

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