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Phytochemical Investigation, Antioxidant and Anti-inflammatory activity of *Nasturtium officinale* W.T. Aiton

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ABSTRACT:

Nasturtium officinale W. T Aiton (Brassicaceae) is a nutritional plant that is often used in traditional systems of medicine. One of its potent uses is the treatment of inflammatory conditions. However, validity of the anti-inflammatory activity has not been scientifically investigated so far. Therefore, the aim of this study was to investigate the anti-inflammatory potential along with antioxidant activity, total phenolic and flavonoids contents. The qualitative chemical screening showed the presence of several phytoconstituents such as proteins, amino acids, carbohydrates, glycosides, alkaloids and others. The different extracts showed significant antioxidant potential as well as total phenolic and flavonoids contents. The hydroalcoholic extract (HAE) possess significant antioxidant potential as shown by DPPH and ABTS methods, respectively, as well as highest total phenolic and flavonoid contents among all the extracts. Further, extract with potent antioxidant potential was subjected for anti-inflammatory activity with the help of the carrageenan-induced paw oedema model. The extract (HAE) at a dose of (750mg/kg) exhibited marked inhibition against carrageenan-induced hind paw oedema. These results provide substantial evidence that the plant Nasturtium officinale has valuable sources of phytochemicals, antioxidant potential and traditional usage as anti-inflammatory agent.

1. INTRODUCTION

Medicinal plants have been used to relieve and cure many ailments from ancient times. In many parts of the world, traditional medicine replaces conventional medicine. Wide range of medicinal plants exhibited antioxidant activity, which is seeking considerable attention among researchers for its involvement in the management of diseases. Plants contain therapeutically active components which are relatively safe and frequently considered to be less/no toxic effects and free from side effects in comparison with the synthetic ones[1]. Medicinal plants derived constituents play a key major role in world health and have long been known to possess various biological effects[2].

Nasturtium officinale W.T. Aiton (*N. officinale*) commonly known as watercress, member of the Brassicaceae family. (Figure 1) It is grown as a perennial, nutritious, and flavorful food that is popular in many countries. *N. officinale* is a richest source of phytochemicals similar to other members of the family. It is found in India, and commonly distributed in the region of Uttarakhand, Madhya Pradesh, Tamil Nadu, Goa, Sikkim, and Punjab. In the Kumaun region of Uttarakhand it is commonly known as Halim[3]. It also contains glucosinolates, tannins, flavonoids, terpenoids and many other glycosides. It is augmented with carotenoids, polyphenols and α-tocopherol. It is a major origin of iron, calcium, iodine and folic acid. It is a rich source of essential vitamins and minerals i.e. lutein and zeaxanthin. It contains vitamin A, vitamin B, vitamin B2 and vitamin C [4]. It has the high content of vitamin C and mineral which makes a remedy for the treatment of various chronic illnesses. This plant is thought to stimulate the appetite and to relieve indigestion; also used in case of chronic bronchitis, and act as a powerful diuretic. It is generally

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consumed as a raw salad and as a vegetable. This plant acts as an appetiser, anti-scorbutic, and stimulant[5].

However, the anti-inflammatory activity of aerial parts of *N. officinale* has not been investigated. Therefore, the aim of the present study was to investigate the anti-inflammatory potential. In addition, we also evaluated the total phenolic and flavonoid contents and antioxidant activity.

Figure1. *Nasturtium officinale* W.T. Aiton in its habitat

2. MATERIALS AND METHODS

2.1 Plant Material

The whole plant was collected from wild area of Ranikhet district of Kumaun region Uttarakhand at an altitude of 1500-4000m during April month. The plant authentication was done by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The voucher specimen number was (Reference number 1243). Plant drug was shade dried (<40°C), coarsely powdered and stored in airtight container.

2.2 Preparation of extract

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 g) was extracted with 250 ml of water, ethanol, and hydro alcohol by the maceration method.

2.3 Preliminary phytochemical screening

The extract was subjected for qualitative preliminary phytochemical screening for the presence of phytochemical as per described standard methods [6].

2.4 Evaluation of total phenolic and flavonoid contents of plant extract

2.4.1Total Phenolic contents

The total phenolic contents of the extracts were estimated by Folin-ciocalteu method. As per the method, 100 µl of the extracts were taken into the text tubes followed by the addition of the 3.0 ml distilled water. Then 0.5 ml of the folin-ciocalteu reagent was added. Then mixed the resultant solution and added 2 ml of 20 % sodium carbonates solution just after 3 min. The solutions were mixed thoroughly and boil for at least 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 phosphomolybdic 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[7,8].

2.4.2 Flavonoid Contents

The aluminium chloride method was used to determine the flavonoid contents (TFC) of the extracts. As per the method, 100 µl of the extracts were taken into the text tubes followed by subsequently addition of the 80% ethanol. Then the aluminium chloride solution (100 µl) was added to each tube 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 for 30min. The absorbance of the resultant

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solution was measured at 415 nm. The flavonoids contents were measured in terms of quercetin and the values expressed as mg quercetin equivalent/g (mg QE/g) on a dry weight basis [7,8].

2.5 Evaluation of antioxidant potential of plant extract

2.5.1 DPPH Free Radical Scavenging Assay

The methanolic solution of DPPH (2 ml of 0.1mmol) was added to the various 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. The percentage free radical scavenging activity of DPPH radicals were calculated and further IC_{50} value was determined[9].

2.5.2 ABTS Free Radical Scavenging Assay

The total antioxidant activity was estimated as per the standard method[10]. The various concentrations (20-100 µl) of the extracts were added into test tubes and then make up 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•+) was produce, when ABTS reacts with the potassium persulphate. ABTS•+ 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•+) to colourless ABTS, which is due to its hydrogen donating availability. The percentage FRSA and IC50 value were calculated.

2.6 Effects of the antioxidant potent extract on carrageenan-induced paw oedema model

2.6.1 Experimental Animals: Young Wistar rat (180-200g) breed in the Central Animal House, Department of Pharmaceutical Sciences, Sir J.C. Bose Technical Campus, Bhimtal, Kumaun University, Nainital (India) was used in the study. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation and kept under standard conditions of a 12 h light/dark cycle with food and water ad libitum in polyacrylic cages. All the experiments were performed between 09.00 and 16.00 h. Experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC) of college (IAEC/ CPCSEA/2021) and carried out as per the guidelines of Committee for Control and Supervision of experimentation on Animals (CPCSEA), Government of India on animal experimentation.

2.6.2 Experimental Protocol and Procedure

Carrageenan-induced paw inflammation was produced according to the method[11]. Wistar rats were randomly divided into 04 equal groups (n = 06) and treated orally in the following manner: each rat in group1 received 1 mL of distilled water (control group), rats in groups 3, 4, received 200, and 750 mg/kg of HAE extract in 1 mL of distilled water respectively, while rats in group 2 received 10 mg/kg of indomethacin, as reference drug. After 1 h, 0.1 mL of carrageenan suspension (carrageenan (1%) suspended in Phosphate Buffered Saline (PBS), pH 7.4) was injected subcutaneously into the plantar surface of left hind paw of all these rats under mild ether anaesthesia. Thereafter, the left hind paw volumes of these rats were measured using the plethysmometer at hourly intervals. The degree of swelling was calculated by the paw volume increase (Vt − V0) where Vt and V0 are the volume of the left hind paw after and before the carrageenan injection respectively. The percent inhibition of inflammation at each interval compared to the controls was calculated for each group as follows:

Percent inhibition = (Vt − V0) in control rats − (Vt − V0) in treated rats × 100/ (Vt − V0) in control rats

2.7 Statistical analysis

Data are given as means ± SEM Statistical comparisons were made using one way ANOVA followed by Duncans Multiple range test. Value $P \leq 0.05$ was considered as significant.

3. Results:

3.1 Percentage of extractive value of extracts

The various solvent extracts were subjected for calculating their % yield, color, and consistency respectively given in Table 1.

Table1. Percentage extractive value of *N. officinale*

Extracts	Color	Consistency	Yield (%w/w)
Aqueous extract (AE)	Brown	Semi-solid	2.90
Ethanol extract (EE)	Brown	Semi-solid	2.10

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Hydroalcoholic extract (HAE)	Greenish brown	Semi-solid	3.20

3.2 Qualitative Phytochemical Screening

The different phytochemical tests were performed on ethanolic, aqueous and hydroalcoholic extracts i.e. (EE, AE, and HAE) of the aerial parts of the plant. The results revealed the presence of reducing sugars, flavanones, tannins, polyphenols, alkaloids, and protein in the plant extracts. (Table 2)

Table2. Preliminary phytochemical screening of various extracts of aerial parts of *N. officinale*

Phytochemical	**Tests**	**Ethanolic (EE)**	**Aqueous (AE)**	**Hydroalcoholic (HAE)**
Amino acids	Ninhydrin	+	+	+
Proteins	Biuret	+	+	+
Carbohydrates	Molisch's	+	+	+
	Fehling's	+	+	+
Glycosides	Legal	+	-	+
	Killer kiliani	+	-	+
	Foam	+	+	+
	Anthraquinones	-	-	-
Steroids	Salkowski reaction	-	-	-
Alkaloids	Mayer's	-	-	-
	Dragendorff's	-	-	+
Flavonoids	Shinoda	+	+	+
Tannins and phenolic compounds	5%FeCl	+	+	+

+ Positive; - Negative

3.3 Total phenolic and flavonoid contents of plant extract

3.3.1Total 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 results showed that HAE extract shows maximum total phenolic content 4.842 mg/g followed by the AE (2.287 mg/g) and EE extract (0.266 mg/g) respectively.

3.3.2 Flavonoids Content

The flavonoid content was determined using quercetin as standard. The TFC was expressed as mg quercetin equivalent (QE)/g on a dry weight basis of the extract. The results showed that HAE extract shows maximum flavonoids content 7.509 mg/g followed by the AE (5.136 mg/g) and EE extract (3.849 mg/g) respectively.

3.4 Antioxidant potential of plant extract

3.4.1 DPPH Free Radical Scavenging Assay

The antioxidant effect was estimated in terms of IC_{50} value. The results revealed that the HAE extract showed the minimum IC_{50} value (0.509 mg/ml) followed by the AE (0.959 mg/ml) and EE extract (2.226 mg/ml) respectively. Among the various extracts, the HAE extracts showed better antioxidant effect followed by the other extracts of the plant. The reference standard used in the study is ascorbic acid (0.00967 mg/ml).

3.4.2 ABTS Free Radical Scavenging Activity Assay

The results revealed that the (HAE) extract showed the minimum IC_{50} value (0.333 mg/ml) followed by the AE (0.660667 mg/ml) and EE extract (1.469 mg/ml) respectively. Among the different extracts, the HAE 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).

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The scavenging ability of HAE extract of *N. officinale* has significant values (0.509 mg/ml for DPPH and 0.333 mg/ml for ABTS) and corresponds to the presence of high quantity of phenolic and flavonoid compounds.

3.5 Effect of potent antioxidant extract (HAE) in Carrageenan-induced paw oedema in rats

In the carrageenan-induced paw oedema model, administration of carrageenan caused significant paw oedema as compared to normal paw in successive time. Pre-treatment with HAE extract (200 mg/kg, 750 mg/kg), standard Indomethacin (10mg/kg) showed significant inhibition in increase paw volume as compared to untreated control group but HAE extract (750mg/kg) showed significant decrease in paw oedema as compared to standard (Diclofenac sodium 10mg/kg). Treatment with HAE extract (750mg/kg) showed significant inhibition in paw volume in 0, 30, 60, 90 minutes as compared to untreated control group. (Table 3) The percentage increase in paw volume at dose HAE extract (200mg/kg) was 21.42%, 26.38%, 44.0 %, 51.31% whereas at 750 mg/kg was only 32.85 %, 41.66 %, 52.0%, 63.1 % and Indomethacin (10mg/kg) was 57.14% , 66.30%, 72.00%, 84.20% at 0,30, 60, 90 minutes as shown in figure2.

Table3. Effect of low dose of HAE (200 mg/kg) and high dose (750mg/kg) of HAE extract in carrageenan induced paw oedema in rats.

Treatment Group	Dose (mg/kg)	0 minutes	30 minutes	60 minutes	90 minutes
Control		0.70±0.003	0.72±0.009	0.75±0.002	0.76±0.006
Indomethacin (Standard)	10	0.30±0.007 [a]	0.24±0.003 [a]	0.21±0.005 [a]	0.12±0.004 [a]
(HAE) Low dose	200	0.55±0.001	0.53±0.03	0.42±0.003	0.37±0.004
(HAE) High dose	750	0.47±0.001 [a,b]	0.42±0.002 [a,b]	0.36±0.005 [a,b]	0.28±0.001 [a,b]

Results are mean ± S.D, (n=6);a $p < 0.05$ vs Control; b $p < 0.05$ vs high dose of extract 750mg/kg

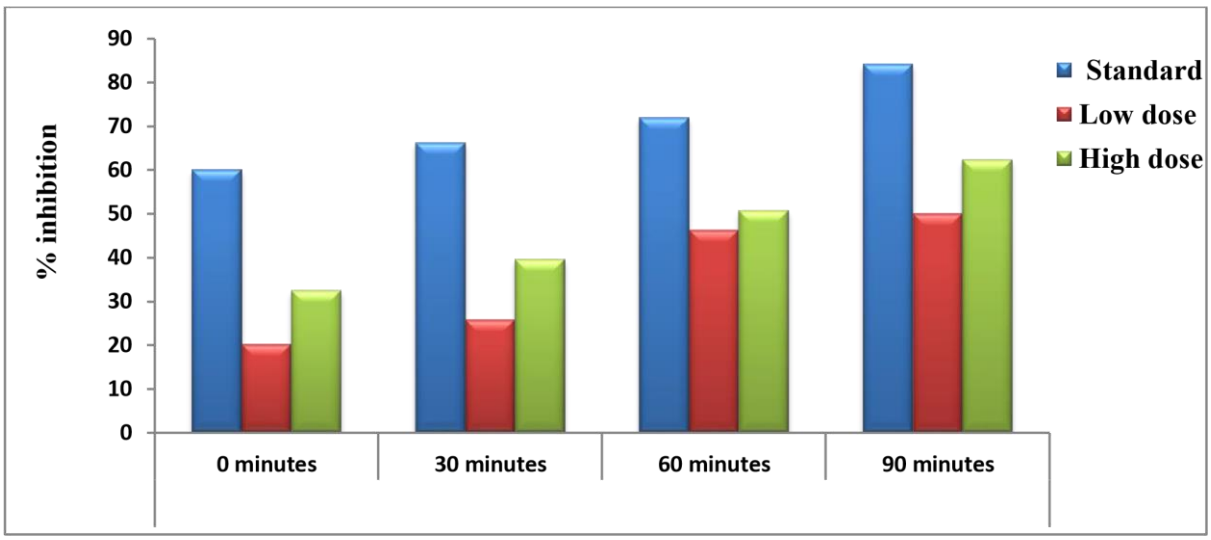

Figure 2: Comparison of % inhibition of standard (Indomethacin) and various doses of hydro-alcoholic extracts (HAE) of whole plant in carrageenan induced paw oedema model.

4. Discussion

In the present study, an attempt has been made to investigate the anti-inflammatory activity of *Nasturtium officinale* by carrageenan-induced paw oedema model. The results of this investigation indicate that antioxidant potent extract (HAE) of *N. officinale* have a marked ability to counter acute inflammation induced by carrageenan.

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The carrageenan-induced paw oedema is commonly used as an experimental model of acute inflammation. The development of this model is believed to be biphasic, of which the first phase is mediated by release of histamine and serotonin while the delayed phase is linked to the neutrophil infiltration, eicosanoid release, production of free radicals and also release of other neutrophil derived mediators[12].

The oedema produced in between early and late phase is thought to be due to the release of kinin-like elements, which later induces the biosynthesis of prostaglandin and other autacoids, which are responsible for the formation of the inflammatory exudates. It is well known that expression of COX-1 and COX-2 are maximal at the early phase and late phase of carrageenan-induced paw oedema respectively[13]. In the carrageenan-induced–induced paw oedema model, *N. officinale* inhibited the oedema in the early and in the late phases of an acute inflammation with maximum inhibitory effect in the late phase.

The phytochemicals which are chargeable for antioxidant effect have attained significant reaction lately for its efficient contribution in prevention of human disorder. Qualitative chemical investigations have shown the presence of phenolics, tannins, and flavonoids. Numerous substances have been suggested to be act as antioxidants. Various phenolic antioxidants such as flavonoids, tannins, and coumarins have been shown to scavenge radicals in a dose dependent manner and therefore are viewed as promising therapeutic drugs for free radical pathologies[14].

Flavonoids and tannins are the major phytoconstituents with antioxidant potential. In recent years, there has been an increased interest in phenolic compounds derived for their possible health benefits. The various biological effects of phenolic compounds are reported to be generally associated with their antioxidant properties of eliminating free radicals[15].

The phenolic compounds present in the sample produced a complex by folin-ciocalteu reagent and the free radical scavenging activities of the phenolic compounds is due to the hydrogen-donating and metal-ion chelating properties. Flavonoids are polyphenolic compounds which act as endogenous antioxidants as a scavenger with several mechanisms[16,17].

The present study shows that significant phenolic and flavonoids compounds were present in each extract of *N. officinale*. The HAE extract contains the highest concentration of phenolic and flavonoids compounds among aqueous (AE) and ethanolic (EE) extract.

During inflammation, the leukocytes and macrophages migrating to the site of injury are known to produce the superoxide radicals (O2−), which in turn mediates the generation of hydrogen peroxide. Furthermore, in the presence of suitable transitional elements, hydrogen peroxide may be transformed to the highly reactive hydroxyl radicals. These radicals can also act as secondary messengers, thereby activating the production of other inflammatory mediators[18].

Many plant extracts having antioxidant properties have been shown to scavenge free radicals and thereby act as anti-inflammatory agents. Polyphenols are plant compounds that can exert significant antioxidant activity, mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[19]. Polyphenols including flavonoids present in the hydroalcoholic extract (HAE) of *N. officinale* may induce the antioxidant properties. Therefore, amalgamations of antioxidant constituents present in the plant extract may also play a major role in arbitrate the anti-inflammatory effects of *N. officinale*.

5. Conclusion

Consequently, the findings suggested that the aerial parts of *Nasturtium officinale* can be used as a direct source of antioxidants. Therefore, it can be used as an accessible source of natural antioxidants with consequent health benefits. Along with it is therapeutically useful for the treatment of inflammatory disorders and rationalizes the traditional usage of this plant as an anti-inflammatory and good nutraceutical agent.

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Conflict of Interest

The author declares that there is no conflict of interest concerning the publication of this work.

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