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Investigation of *In Vitro* Anticancer Potential and Phytochemical Screening of *Nasturtium officinale*

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Abstract: *Nasturtium officinale* (watercress) is commonly known as Haalan in folk traditional language. Traditionally, the plant has been used for treating different ailments. Gluconasturtiin is the main constituent of the plant. This plant contains many nutraceutical benefits and several other major chemical components. Earlier some studies have been reported for the leaves of plant which support antitumor activity. This study supports plant-based diets which have been increasingly recognized for their potential health efficacy as well as life threatening ailments.

The extraction was done with Petroleum ether, chloroform, ethyl acetate and ethanol and investigated for the presence of type of phytoconstituents. Each extract was subjected for *in vitro* anticancer activity. Chloroform extract of *N. officinale* gives potential cytotoxic effect for all cancer cell lines but more effectively against cervical cancer. The study confirmed that *Nasturtium officinale* have significant cytotoxicity specifically for cervical cancer cell line. Hence, further studies will attempt to isolate the bioactive constituents as well as their mechanism of action.

Keywords: *Nasturtium officinale*, Cytotoxicity, Petroleum ether, chloroform, SRB assay

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Introduction

Cancer is considered as one of the life-threatening conditions, which include multiplication of cells and invade from one tissue to the other in the body (Giordano and Petrelli, 2008). Cancer has continuously been the most serious disease in humans around the world due to its high morbidity and mortality rate (Wolff, 2015). Cancer-inducing factors lead to genetic modifications in the expression of oncogenes and antioncogenes (Rose and Ong, 2005). To combat cancer, chemotherapy is a widely used advanced treatment method. These have significant

improvements in anticancer drugs, contributing to better patient care (Sung *et al*., 2021). They often come with adverse side effects (Fallah and Ebrahimi, 2016). Natural products preserve vast pharmacological significance and have been considered as a key source of potential chemotherapeutic treatments. The exploration of natural products continues to be a promising avenue for new and effective treatment cancer (Moradi *et al*., 2017).

Nasturtium officinale (*N. officinale)*, W.T. Aiton belongs to the Brassicaceae family, and has stems that are either creeping or floating along the water. One can find it in brooks, ditches and pond margins, especially in marshy areas. It originally comes from North Africa and Asia (Klimek-Szczykutowicz *et al*., 2019). Watercress is a perennial herb. This plant is not only tasty but also packed with nutrients, rich in vitamin C, vitamin A and α-tocopherol. It has been reported that the leaves of *N. officinale* contain a high concentration of glucosinolates, carotenoids, polyphenols and chlorophyll (Casanova *et al*., 2012). It is medicinally used to treat kidney, stomach, and liver diseases, diabetes, respiratory illnesses, and tuberculosis. Several scientific studies revealed that it has antibacterial, antioxidant, anti-genotoxic, anti-inflammatory and cardio protective properties (Quezada-Lázaro *et al*., 2016).

It is typically sold in fresh form and popularly consumed as a vegetable to enhance the flavours of salads, soups and other recipes. The plant is not only valued for its culinary uses but also for its potential health benefits attributed to phytochemicals, particularly phenolics are considered key bioactive compounds responsible for health advantages associated with a diet rich in plant based foods within the category of secondary plant phenolics.

Flavonoids prevalent in fruits, vegetables and various food products, These compounds serve as active pharmacological agents in many medicinal plants (Palaniswamy and McAvoy, 2001; Yazdanparast *et al*., 2008). An analysis of different plant organs from *N. officinale* such as leaves, stem, flower, and root indicated the presence of phenylalanine derivatives (gluconasturtiin and glucotropaeolin), methionine derivatives (glucoiberin, 7-(methylsulfinyl) heptyl GSL, glucohirsutin, and tryptophan derivatives (glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin) (Aggarwal *et al*., 2019). These diverse compounds contribute to the overall nutritional and potential therapeutic profile of *N. officinale* making it a valuable addition to a healthy diet. The presence of these compounds and their breakdown products—isothiocyanates is utmost important for antioxidant, anticancer, antibacterial, and anti-inflammatory effects of the *N. officinale* extracts (Szczykutowicz *et al*., 2020). The aerial parts of *N. officinale* are the main bases of several medicinally significant phytoconstituents. These chemical constituents protect the human body from oxidative stress by their own capable defense mechanism and curing diseases (Szczykutowicz *et al*., 2020). The objective of the study was to assess *in vitro* cytotoxic activity of *N. officinale* on different cancer cell lines.

Materials and Methods

Plant material:

The plant *N. officinale* was collected from local area of Bhimtal (Uttarakhand, India) and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India (Voucher specimen no.1243). Plant was shade dried (<40ºC), coarsely powdered and stored in airtight container.

Extraction:

The dried plant was coarsely powdered and then extraction was done with Petroleum ether by continuous hot extraction process using Soxhlet's apparatus. The extract was concentrated with rota-evaporator and transferred to a china dish which was previously weighed and dried in a vacuum desiccator. The marc obtained after defatting was then dried in air and further

extraction was carried out with chloroform followed by ethyl acetate and ethanol. The % yield of the extracts was calculated and placed in a desiccator for further use.

Phytochemical screening:

The different solvent extracts (Petroleum ether, chloroform extract, ethyl acetate and alcohol) were qualitatively investigated for the presence of phytochemicals as per described standard methods (Farnsworth, 1966; Harborne, 1998).

Assessment of anticancer activity:

Cell culture:

Breast (MDA-MB-231), lung (HOP-62), colon (COLO-205) and cervical (SiHa) cell lines were cultured separately in liquid medium (DMEM) supplemented 10% fetal bovine serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C. All cell line was purchased from the National Centre for Cell Sciences (Pune, India).

Sulforhodamine B (*SRB*) *assay*:

The anticancer activity was evaluated for the solvent extracts against various cell lines viz. breast (MDA-MB-231), lung (HOP-62), colon (COLO-205), and cervical (SiHa) by using SRB assay. For the present study, cells were inoculated into 96 well microtiter plates in 100 µl at 5000 cells/well. The microtiter plates were incubated at 37°C in a humidified environment with 5% CO_2 in the air following cell inoculation. Following a 24 h incubation period, test samples and the experimental positive control Adriamycin (ADR) were administered in varying doses (10, 20, 40, and 80 µg/ml). Test samples and the standard anticancer drug Adriamycin were first dissolved in 100 mg/ml of dimethyl sulfoxide (DMSO) and then diluted to 1 mg/ml. This is further diluted using a full medium containing test samples to 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml. Aliquots of 10 µl of these different dilutions (e.g. 100–800 µg/ml) were added to the microtiter plate containing 90 µl of the medium, resulting in the needed final concentrations i.e., 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. After the addition of these concentrations, plates were incubated at standard conditions for 48 h, and the assay terminated by adding chilled trichloroacetic acid (TCA). Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 °C. The supernatant was discarded and we washed the plates five times with tap water and air-dried. After adding 50 µl of 0.4% (w/v) sulforhodamine B (SRB) solution in 1% acetic acid to each well, the plates were allowed to stand at room temperature for 20 min. Following staining, the unbound dye was extracted, and any remaining dye was eliminated by repeatedly washing with 1% acetic acid for five times. The plates were then let to air dry. The bound stain was later eluted with a 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with a 690 nm reference wavelength (Vichai *et al*., 2006). The cytotoxicity of the tested drugs on each cell line was reflected by GI_{50} in the data.

We calculated these parameters as GI_{50} = growth inhibition of 50% (GI_{50}) calculated from [(Ti − Tz)/ (C − Tz)] × 100 = 50, drug concentration resulted in a 50% reduction in the net protein increase.

Statistical analysis:

All the data expressed as mean ± SEM.

Results

Percentage yield:

The percentage yield and characterization of solvent extracts (NOPE -- *N. officinale* Petroleum ether extract; NOCE -- *N. officinale* chloroform extract; NOEE -- *N. officinale* ethanol extract and NOEA -- *N. officinale* ethyl acetate) are depicted in Table 1.

Phytochemical analysis:

Phytochemical screening was done for solvent extracts i.e. NOPE, NOCE, NOEE and NOAE of the aerial parts of the plant. The findings showed that the plant extracts contained protein, alkaloids,

Table 1: Percentage yield and color of various solvent extracts from *N. officinale*

Extracts	Color	Odor	Consistency	Yield (%w/w)
NOPE	Pale yellow	Odorless	Semi-solid	22.37
NOCE	Green	Odorless	Semi-solid	21.72
NOEE	Black	Odorless	Semi-solid	20.90
NOAE	Dark black	Odorless	Semi-solid	18.69

Table 2: Phytochemical analysis of various extracts

Phytochemicals	Tests	NOPE	NOCE	NOEE	NOAE
Amino acids	Ninhydrin test	+	-	-	+
Proteins	Biuret test	+	-	-	+
	Millon's test	+	-	-	+
Carbohydrates	Molisch's test	-	-	-	+
	Fehling's test	-	-	-	+
Steroids	Salkowski Reaction	-	-	-	-
Alkaloids	Mayer's test	-	+	-	-
	Dragendroff's test	-	+	-	-
	Hager's test	-	+	-	-
Glycosides	Legal	-	-	-	+
	Keller kiliani Test	-	-	-	+
	Borntrager's test	-	-	-	-
	Foam test	-	-	-	+
Flavonoids	Shinoda's test	-	-	+	-
Phenolic compounds	$FeCl_3$ test	-	-	+	+
	Lead acetate solution test	-	-	+	+

+ Present; - Absent

tannins, polyphenols, flavanones, and reducing sugars. Detailed reports are listed in Table 2.

Anticancer activity:

The *in vitro* anticancer activity of solvent extracts i.e. NOPE, NOCE, NOEE and NOAE of the aerial parts of *N. officinale* are reported in terms of % GI_{50} and the data are shown in Table 3 and Figure 1.

Discussion

Cancer is the second leading cause of death globally, claiming an estimated 9.6 million deaths in 2018 alone; shockingly about 1 in 6 deaths globally can be attributed to cancer. What's particularly concerning is that around 70% of these deaths occur in low and middle-income countries. However, amidst these alarming statistics, these lies hope advances in technology and our understanding of cancer have opened up opportunities to combat this disease more effectively (Zhang *et al*., 2020). Interestingly between 1981 and 2019, when new chemical entities were approved as drugs, only about a quarter were purely synthetic compounds. The majority of these new drugs were actually derived from medicinal plants (Newman and Cragg, 2020). This highlights the crucial role that herbal plants play in the development of anticancer drugs. Indeed herbal plants have significantly contributed to production of anticancer drugs. Many natural substances in these plants possess

Table 3: *In vitro* cytotoxic profile of various solvent extracts

Plant	Cell lines	Sample code	Anti-cancer cell line activity GI_{50}
N. officinale	MDA-MB-231	NOPE	>80
		NOCE	NE
		NOEE	>80
		NOAE	NE
		ADR	<10
	HOP-62	NOPE	>80
		NOCE	NE
		NOEE	>80
		NOAE	NE
		ADR	<10
	Colo-205	NOPE	>80
		NOCE	NE
		NOEE	>80
		NOAE	NE
		ADR	<10
	SiHa	NOPE	>80
		NOCE	19.5
		NOEE	>80
		NOAE	NE
		ADR	<10

Each value represents the mean of three independent experiments; NE= Non evaluable data; GI_{50} = concentration of drug causing 50% inhibition of cell growth; ADR = Adriamycin (Positive control)

properties that can act as antioxidants, cancer preventive agents or even agents that directly combat tumors (Mut-Salud, 2016). Here, we explored the anticancer activity of *N. officinale* various solvent extracts i.e. NOPE, NOCE, NOEE and NOAE against various human cancer cells such as breast (MDA-MB-231), lung (HOP-62), colon (COLO-205), and cervical (SiHa).

Nasturtium officinale is rare aquatic or semi-aquatic plant with rich chemical composition, contributing to its diverse biological activity. Recent pharmacological studies have provided its health-promoting effects. The percentage yield of extracts from *N. officinale* varies depending on the plant parts and species as well as solvent systems used for the extraction. The different plant part contains varying levels of secondary metabolites, which are compounds responsible for many of the plant's beneficial effects. Our research finding suggests that these crude extracts may contain

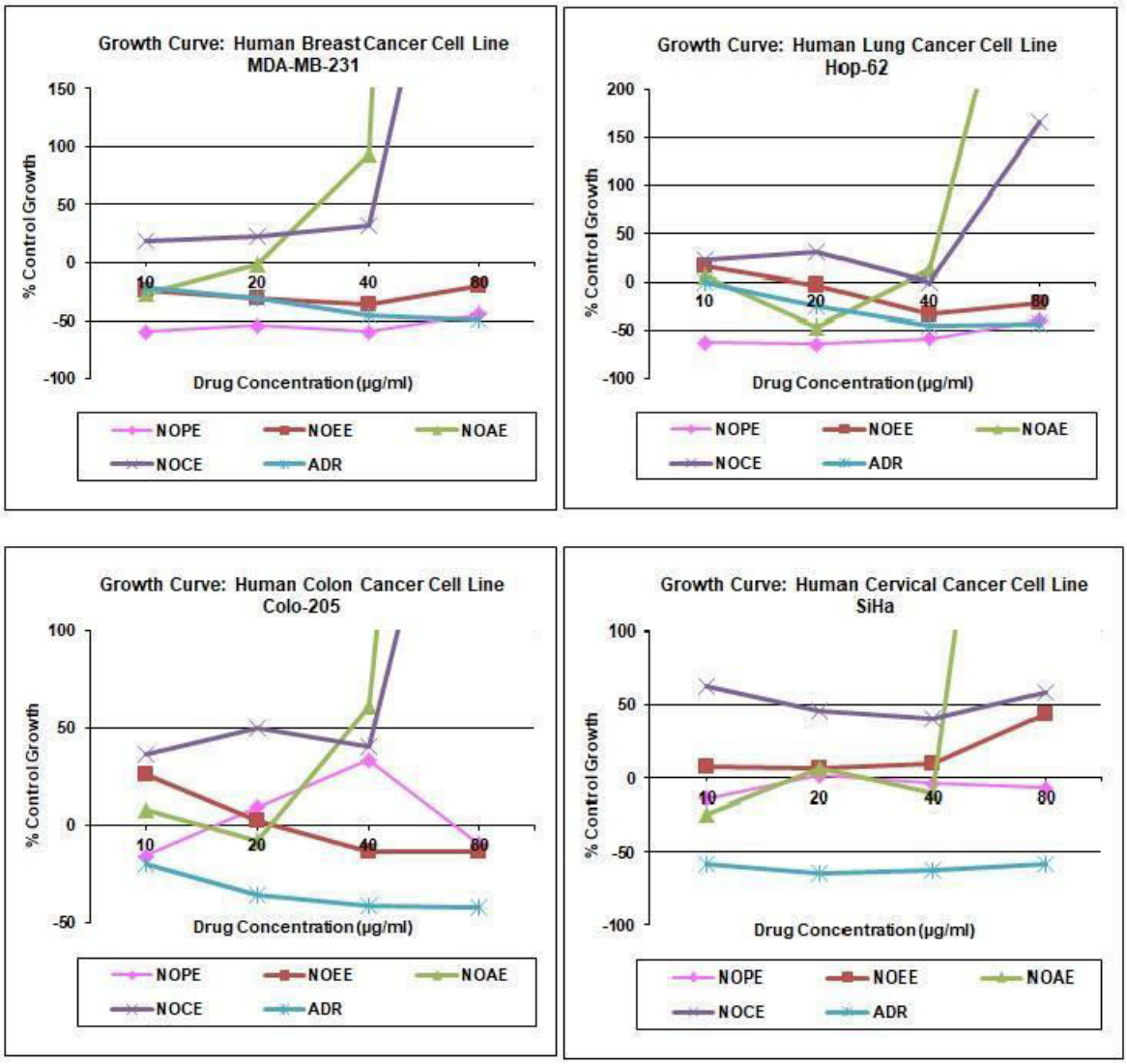

Fig. 1: Impact of various solvent extracts on cell survival.

active compounds capable of combating cancer. This implies the potential for *N. officinale* to serve as a source of anticancer agents, highlighting its significance in medical research and potential application in cancer treatments. In this regard, different solvent extracts i.e. NOPE, NOCE, NOEE, and NOAE were subsequently screened for certain chemical components such as alkaloids, flavonoids, glycosides, and tannins etc. by phytochemical investigation. The phytochemical analysis revealed that most phytoconstituents are present in extracts such as glycosides, saponins, flavanones, tannins, polyphenols and alkaloids. All phytochemicals observed in our study have been previously reported about their anti-cancer activity in several studies (Beutler *et al*., 1998; Chung *et al*., 1998; Kuntz *et al*., 1999; Sun *et al*., 2009; Podolak *et al*., 2010; Fraga *et al*., 2019).

The anticancer screening of various solvent extracts of *N. officinale* was tested using sulforhodamine–B (SRB) assay against cancer cell lines (breast (MDA-MB-231), lung (HOP-62), colon (COLO-205), and cervical (SiHa)) of different histological origins. In the current study, chloroform extract (NOCE) showed more cytotoxicity potency on cervical cancer cell line (SiHa) (GI_{50} = 19.5 µg/ml) when compared to the other cell lines. *N. officinale* also known as

watercress has properties that can fight cancer, this means it could potentially become a powerful ingredient in medicine used to treat cancer. The extracts from watercress might be able to kill the cancer cells while protecting healthy cells.

Conclusion

This study found that *Nasturtium officinale* W.T. Aiton has strong ability to kill cancer cells especially those from cervical cancer according to the results from the sulforhodamine-B analysis. However, more research is needed to figure out which specific compounds in watercress are responsible for the cytotoxic activity, which is in progress in our laboratory. These results suggest that *N. officinale* is a promising source of active compounds against cancer. These findings suggest that watercress could be a valuable source of new and better cancer fighting medicine made from plants.

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References

Aggarwal M, Saxena R and Asif N. (2019) p53 mutant-type in human prostate cancer cells determines the sensitivity to phenethyl isothiocyanate induced growth inhibition. J Exp Clin Cancer Res. 38: 307.

Beutler JA, Hame E, Vlietinck AJ, Haemers A, Rajan P and Roitman JN. (1998) Structure-activity requirements for flavone cytotoxicity and binding to tubulin. J med Chem. 41(13): 2333-2338.

Casanova NA, Ariagno JI, Lopez Nigro MM, Mendeluk GR, Gette MDA, Petenatti, E, Palaoro LA and Carballo MA. (2012) In vivo antigenotoxic activity of watercress juice (Nasturtium officinale) against induced DNA damage. J Appl Toxicol. 33(9): 880-885.

Chung KT, Wong TY, Wei CI, Huang YW and Lin Y. (1998) Tannins and human health: A review. Crit Rev Food Sci Nutr. 38: 421-464.

Fallah N and Ebrahimi S. (2016) The anticancer effect of watercress (*Rorripa Nasturtium Aquaticum*) extract on breast cancer cells. Zahedan J Res Med Sci. 18: e2725.

Farnsworth NR. (1966) Biological and phytochemical screening of plants. J Pharm Sci. 55(3):225-276.

Fraga CG, Crof KD, Kennedy DO and Tomás-Barberán FA. (2019) The effects of polyphenols and other bioactives on human health. Food Funct. 10: 514-528.

Giordano S and Petrelli A. (2008) From single- to multi-target drugs in cancer therapy: When aspecifically becomes an advantage. Curr Med Chem. 15: 422.

Harborne AJ. (1998) Phytochemical methods a guide to modern techniques of plant analysis. In: Phytochemical Methods A Guide to Modern Techniques of Plant Analysis, (ed.) Harborne A.J., ISBN: 978-0412572708.

Klimek-Szczykutowicz M, Szopa A, Blicharska E, Dziurka M, Komsta L and Ekiert H. (2019) Bioaccumulation of selected macro- and microelements and their impact on antioxidant properties and accumulation of glucosinolates and phenolic acids in in vitro cultures of *Nasturtium officinale* (watercress) microshoots. Food Chem. 300: 125184.

Kuntz S, Wenzel U and Daniel H. (1999) Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. Eur J Nutr. 38(3):133-142.

Moradi R, Ebrahimi S, Taravati A, Asrardel F, Khorasani HR and Aghajanpour M. (2017) Cytotoxic effect of hydroalcoholic extract of Rorripa Nasturtium Aquaticum on hela cell line. Int Biol Biomed J. 3: 73-79.

Mut-Salud N. (2016) Antioxidant intake and antitumor therapy: Toward nutritional recommendations for optimal results. Oxid Med Cell Longev. https://doi.org/10.1155/2016/6719534.

Newman DJ and Cragg GM. (2020) Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 83: 770-803.

Palaniswamy UR and McAvoy RJ. (2001) Watercress: A salad crop with chemopreventive potential. Hort Technology 11(4): 622-626.

Podolak I, Galanty A and Sobolewska D. (2010) Saponins as cytotoxic agents: a review. Phytochem Rev. 9(3): 425-474.

Quezada-Lázaro R, Fernández-Zuniga EA, García A, González EG, Alvarez L and Camacho-Corona, MDR. (2016) Antimycobacterial compounds from *Nasturtium officinale*. Afr J Tradit Complement Altern Med. 13(2): 31-34.

Rose Huang PQ, Ong CN and Whiteman M. (2005) Broccoli and Watercress suppress matrix metalloproteinase-9 activity and invasiveness of human MDA-MB-2 31 breast cancer cells. Toxicol

Appl Pharmacol. 209(2): 105-113.

Sun Y, Xun K, Wang Y and Chen X. (2009) A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anti-Cancer Drugs 20(9): 757-769.

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomatara I, Jemal A and Bray F. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 71(3): 209-249.

Szczykutowicz MK, Dziurka M, Blazevic I, Dulovic A, Granica S, Glowniak IK, Ekiert H and Szopa A. (2020) Phytochemical and biological activity studies on *Nasturtium officinale* (Watercress) microshoot cultures grown in RITA® temporary immersion systems. Molecules 25: 5257.

Vichai V, Kirtikara K and Sulforhodamine B. (2006) Colorimetric assay for cytotoxicity screening. Nat Protoc. 1(3):1112-1116.

Wolff AC. (2015) Long term side effects of adjuvant chemotherapy in patients with early breast cancer. 24(1): 23-24.

Yazdanparast R, Seifollah Bahramikia S and Ardestani A. (2008) *Nasturtium officinale* reduces oxidative stress and enhances antioxidant capacity in hypercholesterolaemic rats. Chemico Biological Interactions 172: 176-184.

Zhang Z, Zhou L, Xie N, Nice EC, Zhang T, Cui Y and Huang C. (2020) Overcoming cancer therapeutic bottleneck by drug repurposing. Signal Transduct Target Ther. 5: 113.