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## Exploring The Potential Biological Activities of Watercress (*Nasturtium Officinale*) Extract in Vitro

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### Abstract

*Nasturtium officinale*, commonly known as watercress, is rich in various biologically active phenolic compounds, which enable it to be used for the treatment of various chronic diseases. The primary active phytoconstituents in watercress leaves and stems include total polyphenolic compounds, total condensed tannins, and total flavonoids, which were assessed in various extracts, such as aqueous and methanolic, in this study. A variety of in vitro biological activities were assessed, including antioxidant activities such as total antioxidant activity (TAC) and iron-reducing power (IRP), as well as scavenging activities measured against 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH). Additionally, anti-diabetic activities were measured against the actions of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes, while anti-Alzheimer activities were assessed against the activity of acetylcholinesterase (AChE), and anti-arthritic activities were measured against protein denaturation and the activity of proteinase enzyme. Furthermore, anti-inflammatory activities were evaluated against the actions of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and 5-Lipoxygenase (5-LOX) enzymes. This study concluded that, compared to the other extracts under study, the methanolic extract of watercress exhibited the highest in vitro biological activity. As a result, it may prove to be a useful natural substance in the development of novel treatments for oxidative stress-related disorders.

**Keywords:** *Nasturtium officinale*, Antidiabetic Activity, Acetylcholinesterase (AChE), Antiarthritic Activity, Cyclooxygenases

### 1. Introduction

The basis of both nature conservation and our knowledge of humans are the long-standing relationship and interactions between humans and plants [1, 17]. The plant known as *Nasturtium officinale*, *N. officinale* (Robert Brown, R. Br), is an aquatic or semi-aquatic member of the Brassicaceae family [2]. It is also known by other Latin names, including *Rorippa officinalis* and *Nasturtium aquaticum*. Several pharmacological stud-

ies have been carried out on the herb *N. officinale*, which has been used for a long time in traditional medicine [3–5]. Studies have demonstrated that the plant's antioxidant, anticancer, antibacterial, and cardioprotective properties are attributed to the presence of glucosinolates, isothiocyanates, polyphenols (such as flavonoids, phenolic acids, and proanthocyanidins), terpenes (including carotenoids), vitamins (B1, B2, B3, B6, E, and C), and bioelements [6]. Because of its high concentration of vitamins, folic acid, glucosinolates, iodine, iron, protein, and especially calcium and sulfur compounds, which not only contribute to its distinctive odor but also enhance its nutri-

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tional value, therefore, it is considered a valuable source of vitamins [7]. These components have the potential to either enhance or stimulate the immune response [8, 9]. *Nasturtium officinale*, commonly known as watercress, is traditionally used in medicine to treat various conditions such as diabetes, bronchitis, abdominal pain, asthma, inflammation, blood purification, chest pain, and bleeding cessation. It also included liver health, bile elimination, gallbladder support, kidney health, lung health, throat expectorant, skin conditions, facial scars, iron deficiency, and digestion [10]. In addition, its anti-ulcerogenic actions [11] and anti-tuberculosis, anti-diabetic, cardioprotective, and hepatoprotective properties are emphasized [12]. Through scanned literature reviews, no *in vitro* research has been conducted on the biological activities of different watercress extracts. Therefore, this study aimed to investigate the biological properties of various extracts of this plant, including aqueous and methanolic, through *in vitro* experiments.

## 2. Material and Methods

### 2.1. Preparation of plant extract

The stems and leaves of watercress were gathered, dried, and then sliced. The aqueous and alcoholic (methanolic) extracts were prepared based on the previous study by Zen et al. [13].

### 2.2. Watercress extracts *in vitro*

#### 2.2.1. Main phytoconstituents measurement

All prepared extracts were measured for total polyphenolic compounds (mg gallic acid/100g), total condensed tannins ( $\mu\text{g}/\text{mL}$ ), and total flavonoid contents (mg quercetin/100g) according to methods suggested by Singleton and Rossi [14], Broadhurst and Jones [15] and Arvouet-Grand et al. [16].

### 2.3. Biological activity *in vitro*

#### 2.3.1. Antioxidant activities

The antioxidant activities were evaluated in prepared leaves and stem extracts; the TAC was determined using the protocol proposed by Prieto et al. [17], while the IRP ( $\mu\text{g}/\text{mL}$ ) was determined using the procedure recommended by Oyaizu [18]. Ascorbic acid used as a reference.

#### 2.3.2. Scavenging activity

The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity was assessed using the method described by Rahman et al. [19]. Ascorbic acid served as a positive control at the same concentration. The amount of the DPPH free radical that was suppressed was calculated. The median inhibitory concentration ( $\text{IC}_{50}$ ) for every tested sample can be determined by graphing a range of sample values against the DPPH inhibition percentage. The method of Arnao et al. [20] was used for the determination of the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test. The samples' scavenging abilities were compared to that of ascorbic acid. The amount of the ABTS radical that was inhibited was determined. By plotting a curve with different sample concentrations against the percentage of ABTS inhibition, the  $\text{IC}_{50}$  for each tested substance was calculated.

#### 2.3.3. Anti-diabetic activity

The reference drug employed in this experiment was acarbose, and the percentages of inhibition (%) of the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase were calculated using the methods demonstrated by Wickramaratne et al. [21] and Pistia-Brueggeman and Hollingsworth [22], respectively. Plotting a range of sample concentrations against the percentage of enzyme inhibition allowed the  $\text{IC}_{50}$  of each tested substance to be determined.

#### 2.3.4. Anti-Alzheimer's activity

Ellman's technique [23] was utilized to calculate the percentage of inhibition (%) of the acetylcholinesterase (AChE) enzyme in this experiment, with donepezil serving as the reference medication. The  $\text{IC}_{50}$  of each tested extract was calculated by drawing a curve of sample concentrations versus the percentage of AChE inhibition.

#### 2.3.5. Anti-arthritis activity

This experiment synthesized the reference medication (non-steroidal anti-inflammatory medicine), diclofenac sodium, using a method suggested by Meera et al. [24], to determine the

percentage (%) of inhibition for both protein denaturation and proteinase inhibition according to the methods demonstrated by Das and Suresh Kumar [25] and Oyedapo and Famurewa [26], respectively. The  $IC_{50}$  for each tested sample can be calculated by drawing a curve with a range of sample concentrations vs the percentage of proteinase inhibition.

#### 2.3.6. Anti-inflammatory activity

The ovine/human isoenzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) were inhibited to evaluate their in vitro anti-inflammatory properties. The inhibition percentages of COX-1 and COX-2 were calculated using the COX-1 and COX-2 kit based on the protocol suggested by Alaa et al. [27] and compared to indomethacin as a standard non-steroidal anti-inflammatory drug. The inhibition percentages of 5-Lipoxygenase (5-LOX) were calculated using the 5-LOX kit based on the protocol demonstrated by Huang et al. [28] and compared to Zileuton as a standard drug. The  $IC_{50}$  was calculated using linear regression.

#### 2.3.7. Cytotoxic activity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to evaluate the cytotoxic activities against human hepatocellular (HEPG-2) and colon cancer (CACO-2) cells following the protocols described by Mosmann [29] and Vichai and Kirtikara [30], respectively. The results were compared to doxorubicin as a standard drug. The percentage of change in viability was calculated according to formula:  $(\text{Reading of extract} / \text{Reading of negative control} - 1) \times 100$ . A probit analysis was utilized to determine the percent of cell growth inhibition (%) and  $IC_{50}$ .

#### 2.4. Statistical analysis

The data obtained were analyzed using a one-way analysis of variance test (one-way ANOVA), followed by the Bonferroni test. The results were presented as mean  $\pm$  standard error (SE). Statistical significance was determined at a "p" value of less than 0.05. The methanolic extract was compared to the aqueous extract of each plant part

### 3. Results and discussion

#### 3.1. Phytoconstituents

Prior research has indicated that the primary phyto-constituents in watercress extract are polyphenolic chemicals, namely flavonoids and condensed tannins [31, 32]. Consequently, these components were measured throughout the current investigation. Table 1 revealed that the methanol extract of watercress leaves had the highest total polyphenol concentration ( $275.05 \pm 0.64$  mg gallic acid/100 g), total tannin ( $110.02 \pm 0.26$   $\mu\text{g/mL}$ ), and flavonoids ( $62.87 \pm 0.15$  mg quercetin/100 g). The plant stems' total methanolic extract ranks second with  $206.29 \pm 0.48$  mg gallic acid/100 g,  $82.52 \pm 0.19$   $\mu\text{g/mL}$ , and  $47.15 \pm 0.11$  mg quercetin/100 g. These results were consistent with earlier research by Faizy et al. [33], which indicated that the aerial portions of *N. officinale* plants, leaves, and flowers continued higher concentrations of total phenols and flavonoids in the methanolic extract than the stems.

#### 3.2. In vitro biological activities

##### 3.2.1. Antioxidant activity

The primary approach to preventing and treating chronic diseases caused by excessive lipid oxidation and inflammation, such as cancer, atherosclerosis, and rheumatoid arthritis, involves consuming exogenous antioxidants [34]. The measuring of antioxidants TAC and IRP activities in various watercress extracts indicated that the methanolic extract of watercress leaves had significant ( $p \leq 0.05$ ) higher levels of antioxidant activity,  $660.12 \pm 1.54$  mg gallic acid/g and  $385.07 \pm 0.90$   $\mu\text{g/mL}$ , respectively, than the other tested extracts (Table 1). This finding aligns with a previous study [13], which concluded that all watercress extracts, whether aqueous or alcoholic, showed higher activity levels than the control. The inhibition of chain initiation, the binding of transition metal ion catalysts, the disintegration of peroxides, the obstruction of additional hydrogen abstraction, and radical scavenging could explain these differences [35]. Furthermore, these compounds' primary source of antioxidant activity is their capacity to transfer a hydrogen atom to

alkyl peroxy radicals without joining the cycle of peroxidative radical reactions by combining with hydroperoxides or the substrate [36]. Antioxidant activity and IRP have been shown to be correlated; for some chemicals, this correlation has been previously confirmed and considered important indicators [37].

### 3.2.2. Scavenging activity

Antioxidants' primary role is their ability to scavenge radicals, which prevents electron migration and the transfer of hydrogen atoms [38]. Prior study showed that the extract with greater antioxidant activity had lower  $IC_{50}$  values [39]. It has been discovered that the ABTS test is more sensitive than the DPPH assay because the DPPH radical is only involved in hydrogen ( $H^+$ ) transfer DPPH to DPPH-H, whereas the ABTS radical is involved in the electron transfer pathway (ABTS to ABTS) [40]. This study's total methanolic extract of watercress leaves showed significantly ( $p \leq 0.05$ ) higher inhibition percentages against DPPH ( $59.62 \pm 0.03\%$ ) and ABTS radicals ( $74.53 \pm 0.04\%$ ), as shown in Table 2. This is based on the data of the major active ingredients, such as polyphenolic compounds, condensed tannins, and flavonoids mentioned above. Also, the study revealed lower  $IC_{50}$  values against DPPH ( $3.48 \pm 0.01$  mg/mL) and ABTS radicals ( $5.53 \pm 0.02$  mg/mL). As the quantities of the active ingredients increased, the capacity of the extracts to scavenge radicals also increased. This increase could be related to the rise in TAC and IRP, as measured by DPPH and ABTS [41]. The data showed a connection between the phenolic content of watercress and its antioxidant properties. The extract's ability to reduce can be attributed to the presence of functional groups like amino, carboxyl, and hydroxyl groups, which may increase this correlation [42]. In this investigation, ascorbic acid exhibited stronger DPPH and ABTS radical scavenging action at equivalent dosages compared to the other compounds. It is believed to be a common antioxidant that can eliminate free radicals that dissolve in water. It transforms into an ascorbate radical when it donates an electron to a lipid radical, halting the oxidative chain reaction [43].

### 3.2.3. Anti-diabetic activity

The characteristic that distinguishes diabetes mellitus (DM), a chronic metabolic disorder, is elevated glucose levels [44]. Carbohydrates are broken down by  $\alpha$ -amylase into disaccharides and then by  $\alpha$ -glucosidase into monosaccharides. It is believed that the most effective treatment is to reduce the activity of these enzymes to control hyperglycemia [45, 46]. The current study indicated that acarbose was more effective than the methanolic extract of watercress leaves at inhibiting  $\alpha$ -amylase ( $68.42 \pm 0.05\%$ ) and  $\alpha$ -glucosidase activity ( $51.17 \pm 0.01\%$ ) at equal concentrations. The watercress leaf extract demonstrated the significantly ( $p \leq 0.05$ ) higher inhibitory effect on both enzymes ( $61.59 \pm 0.14\%$ ) and  $\alpha$ -glucosidase activity ( $44.69 \pm 0.14\%$ ) (Table 2). The extract's  $IC_{50}$  values against  $\alpha$ -amylase and  $\alpha$ -glucosidase were  $4.25 \pm 0.03$  and  $3.19 \pm 0.01$   $\mu$ g/mL, respectively, as shown in Table 4. The lowest values were recorded. These cyclic compounds may have an affinity for these enzymes, which explains why they inhibit them in vitro [47]. Furthermore, the amount and orientation of the functional groups in the phenolic compounds, as well as the structure-activity relationship, may all be connected to how these enzymes are inhibited [48].

### 3.2.4. Anti-Alzheimer's activity

Alzheimer's disease is a chronic neurological condition that affects cognitive and memory function. It also results in emotional fluctuations and disturbances to logical thought processes, which eventually cause death and cognitive deterioration [49]. Table 2 illustrated that the methanolic extract of watercress leaves had the least inhibitory effect on the activity of the AChE enzyme when compared to the other extracts that were studied.  $52.13 \pm 0.12\%$  inhibited the AChE enzyme, significantly ( $p \leq 0.05$ ) less than the  $67.91 \pm 0.01\%$  efficiency of donepezil, the reference medication. The extract with the lowest  $IC_{50}$  value ( $5.25 \pm 0.09$  mg/mL) outperformed the standard donepezil ( $4.03 \pm 0.07$   $\mu$ g/mL) in terms of anti-Alzheimer's action (Table 2). This was consistent with previous studies that phenolic compounds' structures and their capacity to suppress the activity of the

Table 1: Concentrations of the major bioactive phytoconstituents and the in vitro antioxidant activities in the different watercress *N. officinale* leaves and stem extracts

Sample		Major Phytoconstituents			Antioxidant activity	
		Total polyphenols (mg gallic acid / 100 gm)	Total condensed Tannins ( $\mu\text{g/ml}$ )	Total flavonoid (mg quercetin / 100 g)	TAC (mg gallic acid / gm)	IRP ( $\mu\text{g/ml}$ )
Leaves	Aqueous Ext.	198.04 $\pm$ 0.46	79.21 $\pm$ 0.18	45.27 $\pm$ 0.11	475.29 $\pm$ 1.11	277.25 $\pm$ 0.65
	Methanolic Ext.*	275.05 $\pm$ 0.64 <sup>a</sup>	110.02 $\pm$ 0.26 <sup>a</sup>	62.87 $\pm$ 0.15 <sup>a</sup>	660.12 $\pm$ 1.54 <sup>a</sup>	385.07 $\pm$ 0.90 <sup>a</sup>
Stem	Aqueous Ext.	148.53 $\pm$ 0.35	59.41 $\pm$ 0.14	33.95 $\pm$ 0.08	356.47 $\pm$ 0.83	207.94 $\pm$ 0.48
	Methanolic Ext.	206.29 $\pm$ 0.48 <sup>b</sup>	82.52 $\pm$ 0.19 <sup>b</sup>	47.15 $\pm$ 0.11 <sup>b</sup>	495.09 $\pm$ 1.15 <sup>b</sup>	288.80 $\pm$ 0.67 <sup>b</sup>

\*Denoted the most effective extract. The values were recalculated from n=3/extract and given as mean  $\pm$  SE, a: significant when methanolic extract compared to the aqueous extract of watercress leaves, b: significant when methanolic extract compared to the aqueous extract of watercress stems at  $p \leq 0.05$ .

Table 2: Their in vitro scavenging, anti-diabetic, and anti-Alzheimer activities of the different watercress *N. officinale* leaves and stem extracts.

Sample		Scavenging activity		Anti-diabetic activity		Anti-Alzheimer activity
		DPPH	ABTS	$\alpha$ -amylase Inhibition (%)	$\alpha$ -glucosidase	AChE
Leaves	Aqueous Ext.	51.17 $\pm$ 0.12	63.96 $\pm$ 0.15	41.78 $\pm$ 0.10	30.32 $\pm$ 0.10	38.57 $\pm$ 0.09
	Methanolic Ext.*	59.62 $\pm$ 0.03 <sup>a</sup>	74.53 $\pm$ 0.04 <sup>a</sup>	61.59 $\pm$ 0.14 <sup>a</sup>	44.69 $\pm$ 0.14 <sup>a</sup>	52.13 $\pm$ 0.12 <sup>a</sup>
Stem	Aqueous Ext.	33.26 $\pm$ 0.08	41.58 $\pm$ 0.10	31.34 $\pm$ 0.07	22.74 $\pm$ 0.07	28.93 $\pm$ 0.07
	Methanolic Ext.	46.19 $\pm$ 0.11 <sup>b</sup>	57.74 $\pm$ 0.13 <sup>b</sup>	46.19 $\pm$ 0.11 <sup>b</sup>	33.52 $\pm$ 0.11 <sup>b</sup>	39.10 $\pm$ 0.09 <sup>b</sup>
STD		Ascorbic Acid		Acarbose		Donepezil
		62.44 $\pm$ 0.05	78.17 $\pm$ 0.01	68.42 $\pm$ 0.05	51.17 $\pm$ 0.01	67.91 $\pm$ 0.01

\*Denoted the most effective extract. The values were calculated from n=3/extract and given as mean  $\pm$  SE, a: significant when methanolic extract compared to the aqueous extract of watercress leaves, b: significant when methanolic extract compared to the aqueous extract of watercress stems at  $p \leq 0.05$ .

AChE enzyme are related [50]. The active elements of AChE are further defined by the presence of groups that have a high propensity to bind to the active site of the enzyme, which has one or more "peripheral" anionic sites apart from the choline-binding pocket that contributes to AChE's

substrate inhibitory capabilities [51].

### 3.2.5. Anti-arthritis activity

An autoimmune disease called arthritis is characterized by inflammation. Extra-articular forms of this disabling illness are known to substantially impact one's quality of life [52]. Protein denatura-

Table 3: Thein vitro anti-arthritis and anti-inflammatory activities of the different watercress (*Nasturtium officinale*) leaves and stem extracts.

Sample		Anti-arthritis activity		Anti-inflammatory activity		
		Protein De-naturation	Proteinase	COX-1	COX-2	5-LOX
		Inhibition (%)				
Leaves	Aqueous Ext.	25.82±0.06	23.16±0.06	51.09±0.25	53.34±0.25	38.19±0.25
	Methanolic Ext.*	26.69±0.06	23.94±0.06	51.87±0.25	54.12±0.25	38.97±0.25
Stem	Aqueous Ext.	19.37±0.05	17.37±0.05	45.30±0.24	47.55±0.24	32.40±0.24
	Methanolic Ext.	20.02±0.05	17.95±0.05	45.88±0.24	48.13±0.24	32.98±0.24
STD		Diclofenac Sodium		Indomethacin		Zileuton
		45.17±0.01	43.27±0.01	71.20±0.22	73.45±0.22	58.30±0.22

\*Denoted the most effective extract. The values were calculated from n=3/extract and given as mean ± SE, a: significant when methanolic extract compared to the aqueous extract of watercress leaves, b: significant when methanolic extract compared to the aqueous extract of watercress stems at p≤0.05.

tion leads to the production of autoantigens in the cases of rheumatoid arthritis, cancer, and diabetes, all of which are inflammatory diseases [53]. Both protein denaturation and the proteinase enzyme are responsible for inflammatory disorders such as arthritis. Therefore, it is believed that one potential arthritis therapy strategy is to block them [54]. The data presented in Table 3 exhibited that the watercress leaf methanolic extract exhibited numerically the most inhibitory effect on the enzyme proteinase (23.94±0.06%) and protein denaturation (26.69±0.06%). Diclofenac sodium, on the other hand, showed the least inhibitory effect, suppressing the proteinase enzyme by 43.27±0.01% and protein denaturation by 45.17±0.01%, respectively. As a result, this extract had the lowest IC<sub>50</sub> value (10.47±0.01 mg/mL) for the activity of the proteinase enzyme when compared to the standard (5.79±0.01 μg/mL), which also had a lower IC<sub>50</sub> value (Table 3). Increased scavenging activity against free radicals, which are key contributors to inflammation and arthritis, may be the reason for this [55].

### 3.2.6. Anti-inflammatory activity

Since lipoxygenase (5-LOX) and COX-1 and COX-2 isoforms are required for the metabolism of arachidonic acid, the activities of these well-known pro-inflammatory enzymes are frequently used to evaluate anti-inflammatory agents [56]. The two isoforms of cyclooxygenase (COX), COX-1 and COX-2, differ greatly from one another [57]. The COX-1 and COX-2 isoforms differ in their chemical structures, intracellular locations, and biological roles, although they do not differ functionally [58]. The data from the current study are shown in Table 4, which revealed that all produced compounds inhibited the activity of the COX-1, COX-2, and 5-LOX enzymes. In addition, the results indicated that the watercress leaf methanolic extract had the greatest inhibitory effect on these enzymes' activity when compared to the other extracts. It lowered the activity of COX-1 (51.87±0.25%) and COX-2 (54.12±0.25%) enzymes more than indomethacin, a non-steroidal anti-inflammatory drug, which suppressed the activities of COX-1 and COX-2 enzymes by 71.20±0.22 and 73.45±0.22%, respectively. In relation to the 5-LOX enzyme's activity, this extract reduced the enzyme's activity by 38.97±0.25% as opposed to



Table 5: Thein vitro cytotoxic activities of the different watercress (*Nasturtiumofficinale*) leaves and stem extracts against human colon (CACO-2) and hepatocellular carcinoma (HEPG-2) cell lines.

Sample		Median Inhibitory Concentration (IC <sub>50</sub> ) (µg/mL)	
		CACO-2 cell lines	HEPG-2 cell lines
Leaves	Aqueous Ext.	246.10 ± 2.39	214.00 ± 2.08
	Methanolic Ext.*	86.63 ± 1.67a	75.33 ± 1.45a
Stem	Aqueous Ext.	333.88 ± 2.33	290.33 ± 2.03
	Methanolic Ext.	280.98 ± 2.68b	244.33 ± 2.33b
STD (Doxorubicin)		53.67 ± 2.03	44.67 ± 1.45

\*Denotedthe most effective extract. The values were calculated from n=3/extract andgiven as mean ± SE, a: significant when methanolic extract compared tothe aqueous extract of watercress leaves, b: significant when methanolicextract compared to the aqueous extract of watercress stems at p≤0.05

the matching standard (Zileuton), which reduced the enzyme's activity by 58.30±0.22%. As a result, this extract had the lowest IC<sub>50</sub> values (15.89±0.02, 12.84±0.08, and 17.67±0.03 mg/mL, respectively) against the COX-1, COX-2, and 5-LOX enzymes when compared to the corresponding standards (5.35±0.02, 4.58±0.02, and 7.43±0.01 mg/mL, respectively), which also had lower IC<sub>50</sub> values (Table 4). These findings are in line with previous research, which showed that the inhibition of COX-1, COX-2, and 5-LOX enzyme activities may be related to their antioxidant and scavenging capabilities, perhaps having anti-inflammatory effects [59].

### 3.2.7. Cytotoxic activity

Cell viability assays, which use the MTT assay, are considered essential criteria for assessing cellular response and determining the extract's efficacy against the occurrence and spread of cancer cells [60]. In this study, the in vitro cytotoxic activity of various extracts was tested against CACO-2 and HEPG-2 cells, in comparison to doxorubicin as a standard drug. It was observed that the watercress leaf methanolic extract had the significantly (p≤0.05) higher activity against CACO-2 and HEPG-2 cells, with the lowest IC<sub>50</sub> values of 86.63±1.67 and 75.33±1.45 µg/mL, respectively, compared to doxorubicin, which had lower IC<sub>50</sub> values (53.67±2.03 and 44.67±1.45 µg/mL, respectively).This was consistent with our earlier research [61] and supported by a more recent

study [62], which suggested that increasing total antioxidant capacity, iron-reduction ability, and free radical scavenging activity could be necessary to enhance anticancer effectiveness by lowering cancer cell proliferation.

## 4. Conclusion

The in vitro biological assays carried out on various leaves and stem extracts of watercress showed that the methanolic extract exhibited the highest in vitro biological activity. It may prove to be a valuable natural product in the development of novel treatments for oxidative stress-related disorders. Further research is recommended to evaluate this effective extract for treating chemically induced diseases in rats through in vivo experiments.

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