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NEW INSIGHTS INTO ANTIOXIDANT AND ANTIVIRAL ACTIVITIES OF TWO WILD MEDICINAL PLANTS: *ACHILLEA FRAGRANTISSIMA* AND *NITRARIA RETUSA*

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ABSTRACT

The present work was undertaken to evaluate the polyphenolic content, antioxidant and antiviral properties of two wild medicinal plants; *Achillea fragrantissima and Nitraria retusa*. The crude extracts of both plants were prepared using methanol and ethyl acetate solvents. Generally, total phenolic content ranged from 0.31 to 15.05 mg gallic acid equivalents /g dw, where total flavonoid content varied from 0.23 to 9.30 mg quercetin equivalents /g dw. Methanolic extract of *A. fragrantissima* showed the highest phenolic and flavonoid content compared to *N. retusa*. However, methanolic extract of *N. retusa* showed strong antioxidant activity (IC_{50}= 68 µg ml^{-1}) in the DPPH free radical scavenging assay. Additionally, both *A. fragrantissima and N. retusa* extracts showed antiviral activity against Rotavirus Wa, Adenovirus type 7 and Coxsackievirus B4. Further research is needed to elucidate the active constituents of both plants which may be useful in the development of new and effective antioxidant and antiviral agents.

KEYWORDS: **Antioxidant activity, antiviral, astrovirus, DPPH, ion chelation, rotavirus.**

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INTRODUCTION

Medicinal herbs have the potential for addressing multiple targets with minor side effects, development of low resistance (due to selective pressure of infective agents) and cost effectiveness. Several medicinal plants have been studied for novel antibacterial, antifungal, antioxidant [1] and antiviral [2] properties. Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress [3]. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer, asthma, arthritis, inflammation, mongolism and in the aging process [4]. To inhibit and scavenge the surplus free radicals antioxidants are needed. Although a number of synthetic antioxidants are available, but they have toxic side effects and, therefore, natural antioxidants have become the target of a great number of research studies [5]. Recently, more attention has been given in medicinal plants, including their extracts and isolated pure compounds, of therapeutic potentials as antioxidants in reducing free radical induced tissue injury. Additionally, over 100 enteric virus species cause a wide variety of illnesses in man. These include hepatitis (e.g. Hepatitis A and E), gastroenteritis (e.g. rotavirus, astrovirus, norovirus, and enteric adenoviruses type 40 and 41), meningitis, fever, rash, (e.g. coxsackieviruses group A and B) conjunctivitis (e.g. coxsackievirus group A), myocarditis and may be diabetes (e.g. coxsackievirus group B) as previously reported [6]. However, identifying new plants with high anti-viral activity can be a good source for the progresses in the viral studies. Moreover, till now there are no drugs in the markets against Rotavirus Wa, Adenovirus type 7 and Coxsackievirus B4. So, there is an increasing need for search of new compounds with antiviral activity [7]. Egyptian flora has become an interesting spot to prospect for new chemical leads or hits due to its species diversity. Two medicinal plants were selected for this study (*Achillea fragrantissima and Nitraria retusa*) considering their medicinal properties. *A. fragrantissima* is a desert plant that has been used for many years in traditional medicine in the Arabia region for the treatment of respiratory diseases and gastrointestinal disturbances [8, 9]. Several flavonoids were isolated from *A. fragrantissima* including swertisin 2□□-arabinosideafroside, cirsimaritin, chrysoplenol, cirsiliol, eupatilin-7-methyl ether, isovitexin 4□-methyl ether [10]. Also, *Nitraria retusa*, locally named "Ghardaq", is a perennial species belonging to the family Nitrariaceae [11]. Fresh leaves of *N. retusa* decoction is used in case of poisoning, upset stomach, ulcers, gastritis, enteritis, heartburn, colitis and colonic abdominal pain [12]. The Nitraria genus is known to be rich in alkaloids [13], which are classically classified into three major groups: tripiperidine alkaloids (e.g., schoberine), indole alkaloids (e.g., nitrarine), and spiro alkaloids (e.g., sibirine, nitraramine and 1-pinitraramine). The present work was designed to evaluate total phenolic, total flavonoid contents and antioxidant activities of methanol and ethyl acetate extracts from *A. fragrantissima,* and *N. retusa*. Their inhibitory effect against 3 viruses named; adenovirus type 7, coxsackievirus B4, and rotavirus Wa strains were also studied.

MATERIALS AND METHODS

Plant materials

A. fragrantissima was collected from Wadi Rishrash, Korymat road, Beni Suef Governorate, and was identified by Prof. A.K. Hegazy, Botany Dept., Faculty of Science, Cairo University, Egypt. Whereas, *N. retusa* was collected from El-Ain El-Sokhna, Suez Governorate, and was identified by Dr. S.R. Hussein, Phytochemistry and Plant Systematic Dept., National Research Centre (NRC), Egypt. Voucher specimens were deposited in the herbarium of the National Research Centre (NRC), Egypt.

Preparation of crude extracts

A. fragrantissima and *N. retusa* aerial parts were air-dried in shadow at room temperature, then pulverized to a fine powder. The pulverized samples (50 g from each plant) were macerated

separately in methanol (Me-OH) and ethyl acetate (ETOAC). Maceration was performed with shaking for 24 h. The extracts were filtered and the residues were re-extracted twice with fresh aliquots of the same solvents. Filtrates of each solvent were pooled and evaporated under vacuum to obtain methanolic and ethyl acetate crude extracts of each plant

Determination of total phenolic (TP) content

Total phenolic content was determined as previously described [14]. An aliquot (300 µl) of each plant extract was completed to 3 ml with methanol then mixed thoroughly with 1.0 ml of ten times diluted Folin- Ciocalteu reagent. After 5 minutes, 1 ml of 7.5 % sodium carbonate was added to the mixture. The mixture was allowed to stand in the darkness for 60 min at 30°C. The absorbance of the reaction mixture was measured at 650 nm. The results were expressed as mg of Gallic (GAE) per g of dry weight.

Determination of total flavonoid (TF) content

An aliquot (300 µl) of each plant extract was completed to 1 ml with methanol then mixed thoroughly with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of bi-distill water. After standing for 30 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm [15]. The results were expressed as mg of Quercetin (QUE) per g of dry weight.

Determination of antioxidant activity

DPPH free radical scavenging activity

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) quenching ability was carried out according to our previously published procedure [16]. One milliliter from a 0.1 mM methanol solution of the DPPH radical was mixed to 3 ml of *A. fragrantissima* methanolic and ethyl acetate extracts and *N. retusa* methanolic extract at various concentrations (25, 50, 75 and 100 µg/ml) and *N. retusa* ethyl acetate extract at various concentrations (100, 150, 200 and 250 µg/ml). Discoloration was measured at 517 nm after 30 min of incubation at room temperature in the dark. Butylated hydroxytoluene (BHT) was used as positive control. Measurements were taken in triplicate. The DPPH scavenging effect (%) was calculated using the following equation: DPPH scavenging effect (%) = $[A_{DPPH} - A_S / A_{DPPH}]$ x100 where, A_{DPPH} is the absorbance of the DPPH solution without the extract and A_S is the absorbance of the solution when the extract is added. The extract concentration providing 50% inhibition of radical-scavenging activity (IC_{50}) was calculated and expressed as µg/ml.

Ferrous ion chelating activity

The ferrous ion chelating activity of all crude extracts was determined according to our previously published procedure [17]. Three milliliters of crude extracts at different concentrations, (250, 500, 750 and 1000 µg/ml) of *A. fragrantissima* methanolic and ethyl acetate extracts, (25, 50, 75 and 100 µg/ml) of *N. retusa* methanolic extract and (100, 150, 200 and 250 µg/ml) of *N. retusa* ethyl acetate extract, were added to 60 µl of $FeSO_4$ (2 mM). The reaction was started by adding 100 µl of ferrozine (5 mM). The mixture was shaken vigorously and kept back to stand at room temperature for 10 min. Absorbance of the mixture was measured at 562 nm. EDTA was used as positive control. The Ferrous ion chelating activity (%) was calculated according to the following equation: Ferrous ion chelating activity (%) = $[1 – A_1 / A_0]$ x100 where A_0 was the absorbance of the control (the reaction mixture without the extracts) and A_1 was the absorbance of the reaction mixture in the presence of the extracts. IC_{50} was calculated and expressed as mg/ml.

Total antioxidant activity

One ml of each extract at different concentrations (250, 500, 750 and 1000 µg/ml) was mixed with 3 ml reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated in a water bath at 95°C for 90 min, after cooling at room temperature the absorbance was measured at 695 nm against control (The reaction mixture without the extract) [18]. Gallic acid at different concentrations (100-400 µg/ml) was used as positive control.

The total antioxidant activity was expressed as absorbance of the sample.

Determination of antiviral activity

Cytotoxicity test

Briefly, all extracts (100 mg) were dissolved in 10 ml of ethanol. Decontamination of extracts was done by adding 12 µl of 100x of antibiotic–antimycotic mixture to 500 µl of each extract. Then, bi-fold dilutions were done to 100 µl of original dissolved samples and 100 µl of each dilutions were inoculated in three cell lines; Human epidermoid cancer cells (Hep-2), Monkey African Green kidney cells (MA104), and Buffalo Green Monkey cells (BGM) cell cultures, obtained from the Holding Company for Biological Products & Vaccines VACSERA, Egypt) previously cultured in 96 multi well plates (Greiner-Bio one, Germany) to estimate the non toxic dose of the tested extracts. Cytotoxicity assay was done using cell morphology evaluation by inverted light microscope and cell viability test applying trypan blue dye exclusion method [19, 20].

Cell morphology evaluation by inverted light microscopy

Three cell lines; Human epidermoid cancer cells (Hep-2), Monkey African Green kidney cells (MA104), and Buffalo Green Monkey cells (BGM) at a concentration (2×10^5 cells/ml) were prepared in 96 well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation at 37°C in a humidified 5% (v/v) CO_2 atmosphere cell monolayers were confluent, the medium was removed from each well and replenished with 100 µl of bi-fold dilutions of different tested extracts prepared in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO BRL). For cell controls 100 µl of DMEM without samples was added. All cultures were incubated at 37°C in a humidified 5% (v/v) CO_2 atmosphere for 72 h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding and shrinking, and cytoplasm granulation and vacuolization. Morphological changes were scored [19].

Cell viability assay

It was done by trypan blue dye exclusion method [20]. Hep-2, MA104, and BGM cell cultures (2×10^5 cells/ml) were grown in 12 well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation, the same assay described above for tested extracts cytotoxicity was followed by applying 100 µl of tested extracts dilutions (bifold dilutions) per well. After 72 h the medium was removed, cells were trypsinized and an equal volume of 0.4% (w/v). Trypan blue dye aqueous solution was added to the cell suspension. Viable cells were counted under the phase contrast microscope.

Determination of adenovirus type 7, rotavirus Wa strain, and Coxsackievirus B4 titers using plaque assay

Non toxic crude extracts dilutions were mixed (100µl) with 100µl of different doses of adenovirus type 7 ($1X10^5$, $1X10^6$, $1X10^7$) and ($1X10^4$, $1X10^5$, $1X10^6$) for both rotavirus Wa strain and Coxsackievirus B4. The infectivities of the rotavirus stocks were activated with 10 µg/ml trypsin for 30 min at 37°C. The mixture was incubated for 30 min in 37°C. The inoculation of (100 µl) 10 fold dilutions of treated and untreated Adenovirus type 7, rotavirus Wa strain, and Coxsackievirus B4 was carried out into Hep 2, MA104, and BGM cell lines for adenovirus type 7, rotavirus Wa strain, and Coxsackievirus B4 respectively in 12 multi well plates. After 1 hr of incubation for adsorption at 37°C in a 5% CO_2-water vapor atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 ml of 2X media (Dulbecco´s Modified Eagle Medium, Gibco-BRL (DMEM) plus 1.0 ml 1% agarose was added to each well, 0.5 µg/ml was added to the media-agarose mixture in the case of rotavirus Wa strain and the plates were incubated at 37°C in a 5% CO_2-water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formaline fixation, and the number of plaques counted. The viral titers were then calculated, and expressed as plaque-forming units per milliliter (pfu/ml) as previously reported [21].

Statistical analysis

Statistical analysis was carried out using Microsoft corporation excel program. All experiments were performed in triplicate. Results are presented as a value ± standard deviation of mean (SD).

RESULTS AND DISCUSSION

Total phenolic (TP) and total flavonoid (TF) contents

As shown in Fig. (1), methanolic extract of *A. fragrantissima* exhibited the highest values of total phenolic and total flavonoid contents (15.05±0.98 mg of GAE per g dw and 9.30±0.55 mg of QUE per g dw) respectively as compared with ethyl acetate extract (9.58±0.98 mg of GAE per g dw and 8.93±0.55 mg of QUE per g dw) respectively for the same plant. The present results are in analogous with Shahat et al. [22] who reported that 80% methanol extract of *A. fragrantissima* gave total phenolic content (11.0 mg of GAE per g dw). Also, the total phenolic and flavonoid contents in different species of genus Achillea were reported [23]. It should be noted that, because of polarity differences between solvents, the solubility of the solute into the solvent is expected to be different.

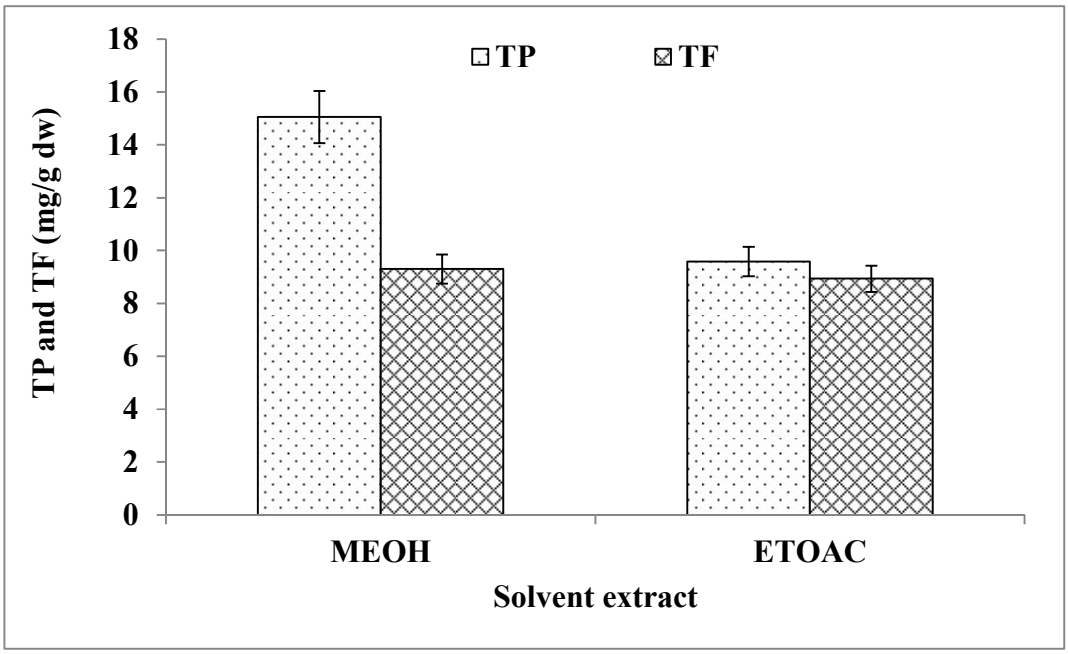

Figure 1

Total phenolic (TP) and total flavonoid (TF) contents in methanolic and ethyl acetate crude extracts of A. fragrantissima. Vertical bars on the columns represent mean ± SD (n = 3).

Likewise, in Fig. (2), *N. retusa* exhibited higher total phenolic and total flavonoid (4.77±0.08 mg of GAE per g dw and 3.49±0.06 mg of QUE per g dw) content respectively in the methanolic extracts than ethyl acetate extracts. Our results showed that the methanol was better solvents for phenolic and flavonoid extraction from aerial parts of *A. fragrantissima* and *N. retusa* compared to ethyl acetate solvent. The present results are in agreement with other similar report [24], the authors stated that, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone. Contrariwise to the present results, the crude methanol extract of *N. retusa* gave total phenolic and total flavonoid contents (46 mg of GAE per g dw and 29 mg of QUE per g dw) lower than that of its ethyl acetate fraction extract which gave (170 mg of GAE per g dw and 60 mg of QUE per g dw) respectively [25].

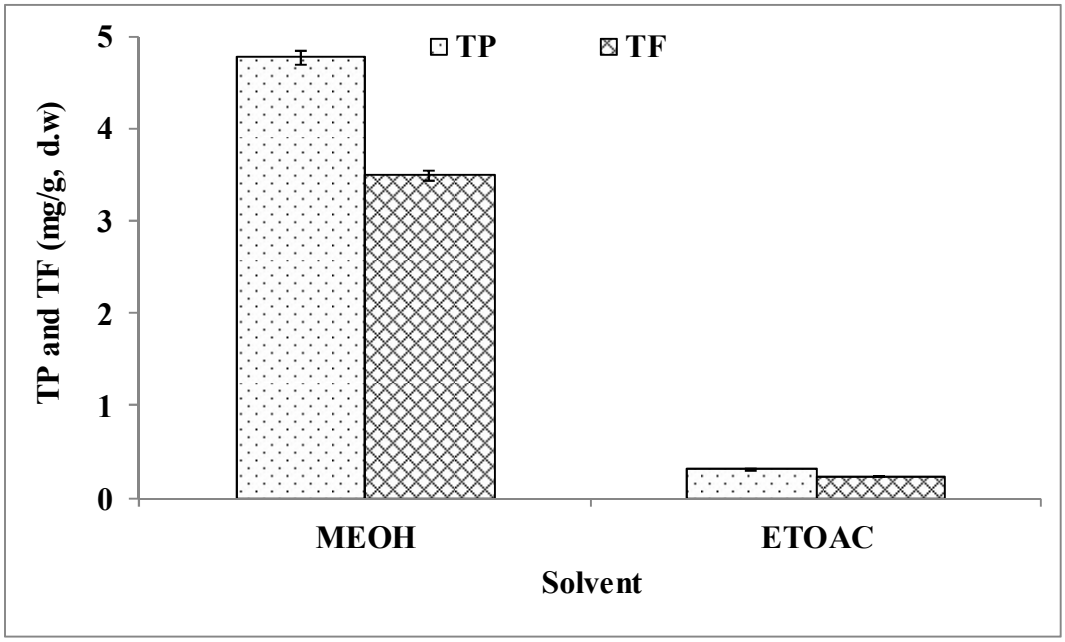

Figure 2
Total phenolic (TP) and total flavonoid (TF) contents in methanolic and ethyl acetate crude extracts of N. retusa. Vertical bars on the columns represent mean ± SD (n = 3).

Antioxidant activities
DPPH radical scavenging activity

DPPH scavenging activity of methanolic and ethyl acetate crude extracts of *A. fragrantissima* increased in a concentration dependent manner as presented in Fig. (3). The methanolic extract exhibited the most potent DPPH scavenging activity (60.48±0.42%) at concentration 100 µg/ml with IC_{50} value (80.99 µg/ml) as compared to the ethyl acetate extract which exhibited 50.07±0.82% activity at the same concentration with IC_{50} value (93.35 µg/ml). The BHT showed potent DPPH scavenging activity with IC_{50} value (70 µg/ml) higher than *A. fragrantissima* methanolic extract. In this concern, the 70% ethanol crude extract of *A. fragrantissima* aerial parts gave lower DPPH scavenging activity with IC_{50} value (35.5 µg/ml) than that of its ethyl acetate fraction extract which gave IC_{50} value (5.4 µg/ml) [26]. Similarly, the DPPH scavenging activity of different species of genus Achillea were also reported [23, 27].

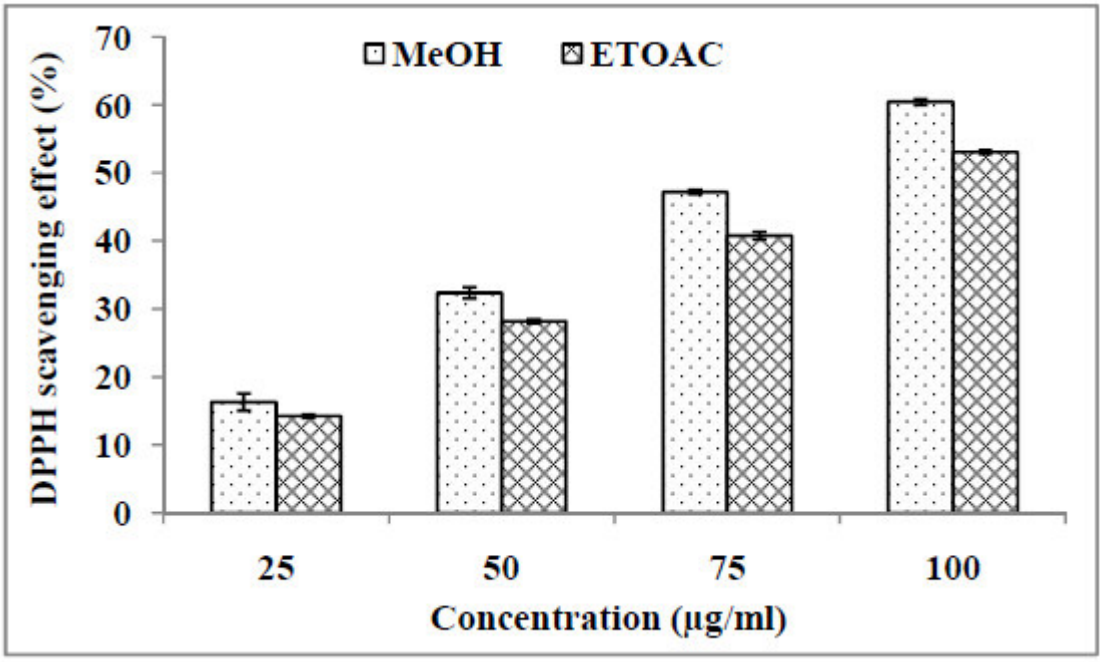

Figure 3
DPPH scavenging activity (%) of methanolic and ethyl acetate crude extracts of A. fragrantissima. Vertical bars on the columns represent mean ± SD (n = 3).

The same pattern of DPPH scavenging activity was found in *N. retusa* crude extracts. The methanolic extract exhibited higher DPPH scavenging activity (69.8±1.3%) than ethyl acetate extract (23.7±1.1%) at a concentration of 100 µg/ml (Fig. 4). The values of IC_{50} were in the following order methanolic extract □ BHT □ ethyl acetate extract with values of 68, 70 and 312 µg/ml respectively. According to the present results, antioxidant activity of both extracts in DPPH assay is dependent on the polarity of extracts. The more polar extract (methanol), had the highest antioxidant activity. Based on the results obtained, it is possible that several compounds of different polarities may contribute to the antioxidative properties of both plant extracts. However, methanolic extracts may include phenolic and hydrox-phenolic compounds with acid, alcohol, sugar or glycoside. Part of the antioxidative activity may be due to these components or flavonoids. In addition, antioxidative activities observed in both solvents extracts could be the synergistic effect of more than two compounds that may be present in the extracts. The difference in the antioxidant activity of extracts may be described by the difference in the total phenolic and flavonoid content [1, 28]. The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen- or electron-donating agents, and metal ion chelating properties [29, 30]

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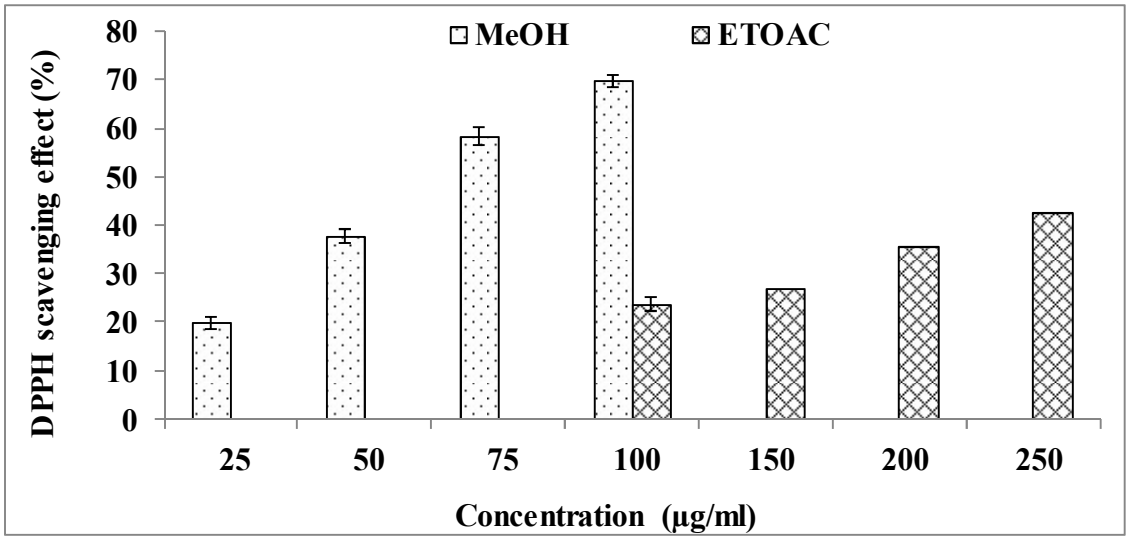

Figure 4

DPPH scavenging activity (%) of methanolic and ethyl acetate crude extracts of N. retusa. Vertical bars on the columns represent mean ± SD (n = 3).

Ferrous ion chelating activity

Generally, all extracts showed different degrees of electron-donating capacity in a concentration-dependent manner. The Fe^{2+} chelating activity of both *A. fragrantissima and N. retusa* methanolic and ethyl acetate extracts was increased with the increase of concentrations (Figs. 5 and 6). In *A. fragrantissima* the methanolic extract exhibited 53.63±0.29% Fe^{2+} chelation activity higher than 36.48±0.54% chelation of ethyl acetate extract at 1000 µg/ml concentration (Fig. 5). The IC_{50} of the Fe^{2+} values for EDTA (positive control), methanolic extract and ethyl acetate extract were 0.028, 0.937 and 1.444 mg/ml, respectively.

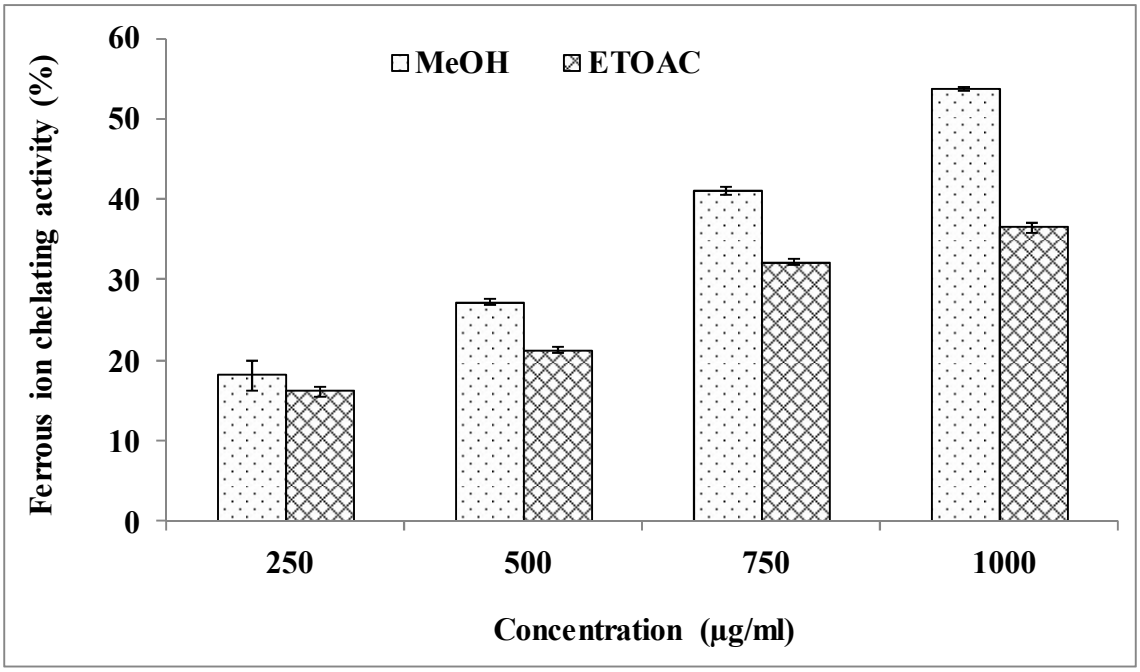

Figure 5

Ferrous ion chelating activity (%) of methanolic and ethyl acetate crude extracts of A. fragrantissima. Vertical bars on the columns represent mean ± SD (n = 3).

In *N. retusa* also the methanolic extract exhibited 31.3±0.95% Fe^{2+} chelation activity higher than 11.8±0.75% chelation of ethyl acetate extract at 100 µg/ml (Fig. 6). The IC_{50} of the Fe^{2+} values for EDTA, methanolic extract and ethyl acetate extract were 0.028, 0.180 and 0.258 mg/ml, respectively. In accordance with DPPH results, the methanolic extracts of both *A. fragrantissima and N. retusa* showed superior Fe^{2+} chelating activity to ethyl acetate extracts, and this may be attributed to its higher contents of both total phenolic and total flavonoids (Figs. 1 and 2). These results are in agreement with those of Shahat et al. [22] who found a correlation between the total phenolic, total flavonoid contents and Fe^{2+} chelating activity. Metal chelating potency of phenolic compounds is dependent upon their unique phenolic structure and the number and location of the hydroxyl groups [31].

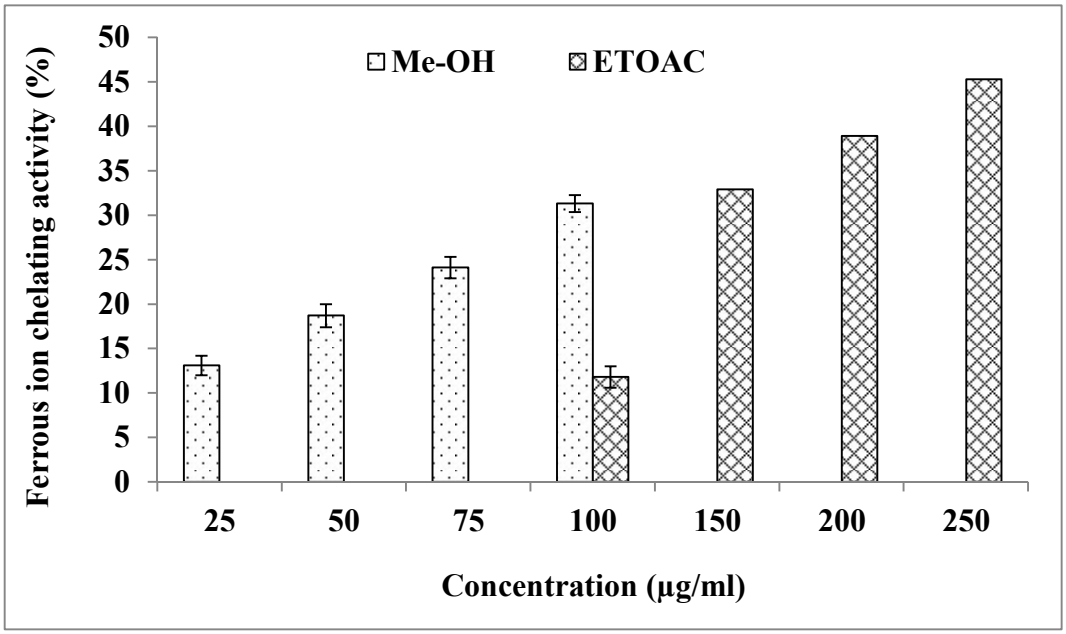

Figure 6

Ferrous ions chelating activity (%) of methanolic and ethyl acetate crude extracts of N. retusa. Vertical bars on the columns represent mean ± SD (n = 3).

Total antioxidant activity

The total antioxidant capacities of the extracts of both plants were quantitatively determined by the formation of phosphomolybdenum complex. This method is based on the reduction of Mo (VI) - Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex, which has maximal absorption at 695 nm. The results of total antioxidant activity of crude extracts of *A. fragrantissima* presented in (Fig. 7) showed dose dependent activity where the activity increased with increasing the concentration. The methanolic extract displayed the highest absorbance (0.978±0.055) as compared to the absorbance (0.869±0.031) of ethyl acetate extract at 1000 µg/ml concentration. While, gallic acid (used as positive control) gave absorbance (0.271) at 200 µg/ml concentration. In this context, Shahat et al. [22] reported that 80% methanol extract of *A. fragrantissima* aerial parts showed remarkable total antioxidant capacity.

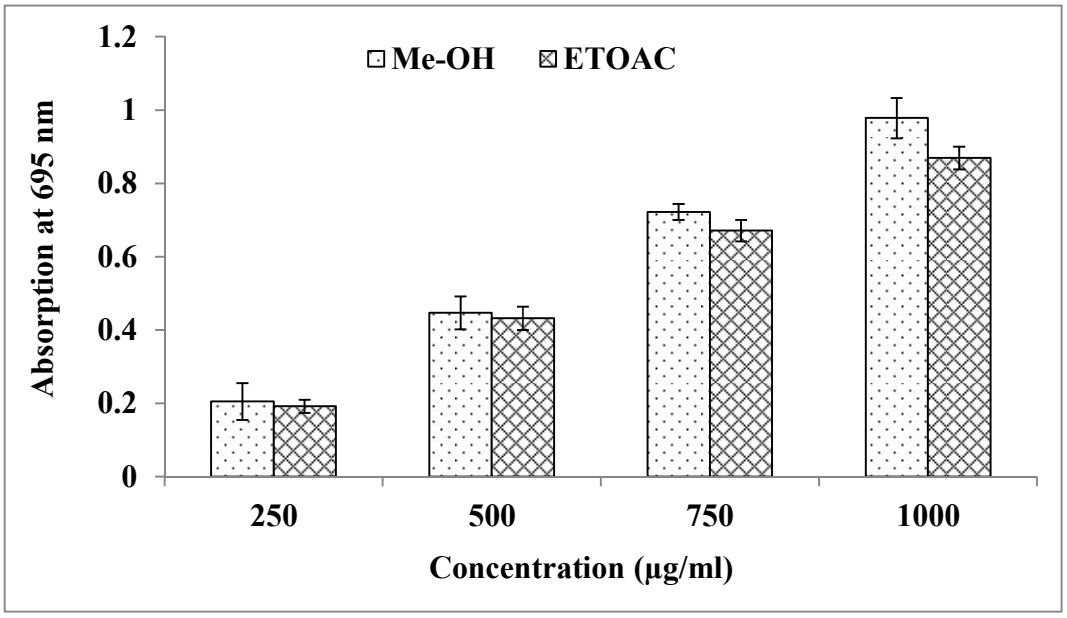

Figure 7
Total antioxidant activity of methanolic and ethyl acetate crude extracts of A. fragrantissima. Vertical bars on the columns represent mean ± SD (n = 3).

The same trends of results were presented in *N. retusa* where, the methanolic extract showed superior total antioxidant activity (0.721±0.43) compared to ethyl acetate extract (0.636±0.24) at 1000 µg/ml concentration (Fig. 8). The present study demonstrated that in both *A. fragrantissima* and *N. retusa* the superiority of total antioxidant activity of methanolic extracts may be attributed to its highest contents of both total phenolic and total flavonoid. Recent studies have shown that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [32, 33].

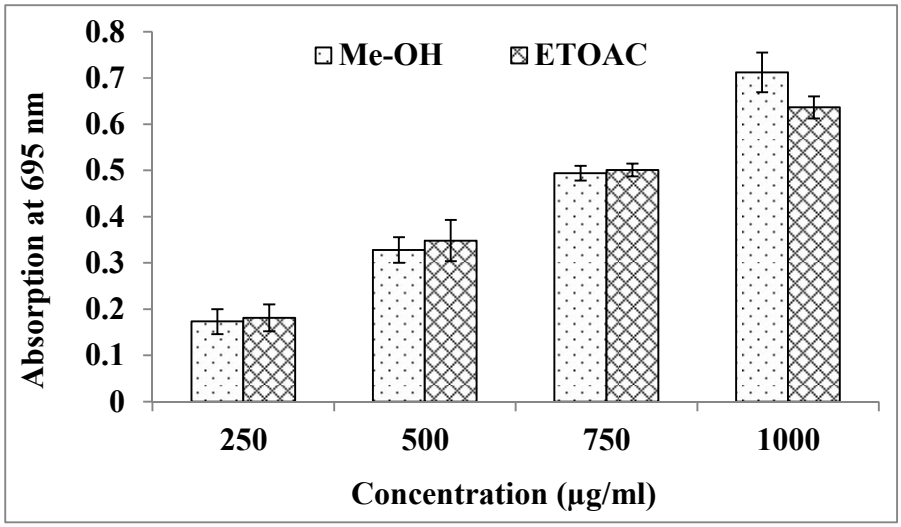

Figure 8
Total antioxidant activity of methanolic and ethyl acetate crude extracts of N. retusa. Vertical bars on the columns represent mean ± SD (n=3).

Cytotoxic effects and antiviral activity of A. fragrantissima and N. retusa extracts

The *A. fragrantissima and N. retusa* (methanol and ethyl acetate) extracts showed different cytotoxic effects on the different cell lines (MA104, Hep 2, and BGM), and the non-toxic doses of the tested extracts were in the following; order *N. retusa* ethyl acetate extract *A. fragrantissima* ethyl acetate extract *A. fragrantissima* methanol extract *N. retusa* methanol extract as presented in Table 1. These cytotoxic effects correlated well with morphological changes recorded during microscopic examination of treated cultures. These changes consisted of cell rounding, appearance of cytoplasmic inclusions and the loss of monolayer confluence as reported previously [19]. In this situation, the aqueous and hydro-alcoholic extracts of *Achillea kellalensis* showed cytotoxic effects on MA104 cell line with 50% viability reduction at 800 µg/ml and 700 µg/ml, respectively [34]. Also, 80% aqueous ethanol extract of *A. fragrantissima* showed non toxic dose (10-100 µg/ml) on VERO cell line [35]. The present work showed that, *A. fragrantissima and N. retusa* (methanol and ethyl acetate) extracts have different ability in reducing viral titres against Rotavirus Wa, Adenovirus type 7 and Coxsackievirus B4. The enteric viruses considered as human pathogens of major clinical interest, since they are associated with high morbidity and globally spread human diseases. As demonstrated in Tables (2, 3 and 4), ethyl acetate extracts of both *A. fragrantissima and N. retusa* exhibited antiviral activity higher than methanol extracts against enteric viruses. The highest percentage of viral reduction (76.7%) was observed with *A. fragrantissima* ethyl acetate extract against Coxsackievirus B4 followed by *N. retusa* ethyl acetate extract exhibited (70%) reduction against the same virus (Table 4) at the non toxic dose of each extract. The antiviral activity of *A. fragrantissima* and *A. kellalensis* against poliomyelitis-1 virus (POLIO) and rotavirus, respectively was reported [34, 35]. The antiviral activity of *A. fragrantissima* and *N. retusa* extracts might be probably due to its contents of phenolic and flavonoids. In our previous work on *N. retusa*, we detected Eugenol in its GC-MS profile [36], however, Eugenol (4-allyl-1-hydroxy-2-methoxybenzene) was tested for antiviral activity against HSV-1 and HSV-2 viruses, and the results demonstrated that, the replication of these viruses was inhibited in the presence of this compound [37]. Moreover, recent studies reported that the antiviral activities of different medicinal plants are attributed to the presence of phenolic and flavonoids [38, 39]. Besides, the highest antiviral activity of ethyl acetate extracts in the present study might be probably due to that ethyl acetate solvent was able to extract most of the non-polar compounds which are mostly of terpenes nature. In this anxiety, Astani et al. [40] mentioned that the sesquiterpenes were able to suppress viral infection by 40-98% and directly inactivate herpes virus by interfering with virion envelope structures or mask viral structures that are necessary for adsorption or entry into host cells. Also, Aref et al. [41] stated that the triterpenes showed activity against HSV-1 and their mode of action was found at all stages of multiplication.

Table 1
Non-toxic doses (mg/ml) of different plant extracts on MA104, Hep2, and BGM cell lines

Plant extracts	Non-toxic doses (mg/ml)		
	MA104	Hep2	BGM
A. fragrantissima (ETOAC).	1.2	1.3	1.4
N. retusa (ETOAC)	1.4	1.4	1.4
A. fragrantissima (Me-OH)	1.0	0.9	1.0
N. retusa (Me-OH)	0.9	0.9	0.9

Table 2

Anti rotavirus Wa strain activity of non-toxic doses* from different plant extracts

Plant extracts	Initial viral titre	Final viral titre	% of reduction	Mean % of reduction
A. fragrantissima (ETOAC)	$1X10^4$	$3X10^3$	70%	66.7%
	$1X10^5$	$3X10^4$	70%	
	$1x10^6$	$4x10^5$	60%	
N. retusa (ETOAC)	$1X10^4$	$5X10^3$	50%	56.7%
	$1X10^5$	$4X10^4$	60%	
	$1x10^6$	$4x10^5$	60%	
A. fragrantissima (Me-OH)	$1X10^4$	$6X10^3$	40%	33.3%
	$1X10^5$	$7X10^4$	30%	
	$1x10^6$	$7x10^5$	30%	
N. retusa (Me-OH)	$1X10^4$	$5X10^3$	50%	50.0%
	$1X10^5$	$5X10^4$	50%	
	$1x10^6$	$5x10^5$	50%	

* *The non-toxic doses was of MA 104 cell lines as presented in Table 1*

Table 3

Anti adenovirus type 7 strain activity of non-toxic doses* from different plant extracts.

Plant extracts	Initial viral titre	Final viral titre	% of reduction	Mean % of reduction
A. fragrantissima (ETOAC)	$1X10^5$	$4X10^4$	60%	53.3%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	
N. retusa (ETOAC)	$1X10^5$	$5X10^4$	50%	50.0%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	
A. fragrantissima (Me-OH)	$1X10^5$	$6X10^4$	40%	23.3%
	$1X10^6$	$8X10^5$	20%	
	$1x10^7$	$9x10^6$	10%	
N. retusa (Me-OH)	$1X10^5$	$5X10^4$	50%	50.0%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	

* *The non-toxic doses was of Hep 2 cell lines as presented in Table 1*

Table 4

Anti Coxsackievirus B4 activity of non-toxic doses* from different plant extracts

Plant extracts	Initial viral titre	Final viral titre	% of reduction	Mean % of reduction
A. fragrantissima (ETOAC)	$1X10^4$	$2X10^3$	80%	76.7%
	$1X10^5$	$2X10^4$	80%	
	$1x10^6$	$3x10^5$	70%	
N. retusa (ETOAC)	$1X10^4$	$3X10^3$	70%	70.0%
	$1X10^5$	$3X10^4$	70%	
	$1x10^6$	$3x10^5$	70%	
A. fragrantissima (Me-OH)	$1X10^4$	$4X10^3$	60%	53.3%
	$1X10^5$	$5X10^4$	50%	
	$1x10^6$	$5x10^5$	50%	
N. retusa (Me-OH)	$1X10^4$	$3X10^3$	70%	66.7%
	$1X10^5$	$3X10^4$	70%	
	$1x10^6$	$3x10^5$	60%	

* *The non-toxic doses was of BGM cell lines as presented in Table 1*

CONCLUSION

The results of the present study revealed that both *A. fragrantissima* and *N. retusa* crude extracts exhibited antioxidant and antiviral activities, which might be attributed to its potent contents of phenolic and flavonoids. This report described for the first time the antiviral activities of both *A. fragrantissima* and *N. retusa* crude extracts against Rotavirus Wa, Adenovirus type 7 and Coxsackievirus B4. Till now, there are no drugs in the markets against Rotavirus Wa, Adenovirus type 7 and Coxsackievirus B4. Therefore, the potent antiviral activities of *A. fragrantissima* and *N. retusa* aerial parts may support their use as a natural source of antiviral substances for the enteric viruses treatments. Also, since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compounds which may promote their use as natural sources of antioxidants and antiviral agents.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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