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Anti-proliferation properties of Egyptian medicinal herbs *Moringa oleifera* and *Vinca rosa*crude extracts against Human colorectal cells

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Abstract

Alternative cancer treatment attracted researchers in the last decades. Plants are enriched in their natural constitutes of various phytochemicals. These compounds include several secondary metabolites belong to different chemical groups. In this research, we extracted leaves of two Egyptian wild plants, *Moringa oleifera* and *Vinca rosa* with 80% ethanol then tested for their biological activity. The extracts showed strong antioxidant and anticancer activities. The antioxidants were evaluated using DPPH and reducing power assays. Moringa and vinca displayed potential antioxidants expressed by IC50 at 55.35 and 185.05 µg/ml, respectively. The present results also indicated that vinca extract exhibited high anticancer potencies against colorectal (Caco-2) with IC50 11.7 µg/ml, while moringa showed lower anticancer activity with IC50 at 25.32 µg/ml. Phenolic (TP) and flavonoids contents were evaluated and fractionated using the HPLC technique and the analysis showed that TP and TF were nearly the same in Moringa and vinca extracts. TP were 161.6 and 164.43 mg gallic acid equivalent/ gm and 137.84 and 141.8671 mg quercetin equivalent/ gm sample, respectively. These compounds may be responsible for the mentioned biological activities. *Moringa oleifera* and *Vinca rosa* might contains specific phytochemical induces apoptosis, for that we tested that gene expression of key pro- and anti-apoptosis markers in Caco2 cells exposed to IC50 which the apoptosis induction was analyzed. We tested the gene expression of various signal transduction key markers controlling apoptosis process, such as p53, Bax, caspase-3 and Bcl-2 key markers. The RTqPCR-results showed that Moringa have potential apoptosis induction compared to *vinca* and respective control.

*Keywords:*Moringa and vinca, Polyphenols, Flavonoids, HPLC Spectrometry, Anticancer, Antioxidants

1. Introduction

Even though cancer medication discoveries and chemotherapeutic agents have made tremendous advances in recent decades, cancer remains a deadly and aggressive disease. The second most common cause of death is cancer worldwide, after coronary artery disease [1]. Cancer therapeutic regimens, including surgery, chemo, and radiotherapy, are based on traditional remedies and herbal medicine. Cancer continues to be a major health issue around the world. Despite medical progress over the last decade, more research into cancer genetics and the application of innovative medication therapies is ongoing. Around 782,000 people are diagnosed with colon cancer [2]. For thousands of years, medicinal plants have been employed in folk medicine as natural products that play an important part in pharmacological biology. The active elements of anticancer medications have been derived from natural materials, according to the World Health Organization (WHO) [3]. Furthermore, contemporary medications have completely or partially similar structures to natural molecules. Because conventional cancer treatments have severe side effects, combining natural compounds like Vinorelbine and Paclitaxel with chemotherapy has shown promising outcomes [4]. *Moringa oleifera* is a Himalayan plant and thrives in both semi-tropical and tropical climates. It is frequently utilized in ethnobotanical science and treats several ailments [5] effectively. Moringa, sometimes known as the "Miracle Tree," has long been used in Ayurvedic treatment in India. The entire Moringa plant is devoured., Especially

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popular are the leaves and pods. *Moringa oleifera* leaves are used as a dietary supplement and boost both the immune system and energy levels. They are also reported to possess antioxidant and anti-inflammatory activities. Moringa is utilized in the preparation of both hot and cold beverages (teas and juices) in many Jamaican households and meals. Moringa has sparked a lot of attention [6,7,8,9]. *vinca rosa* (Apocynaceae) has long been used to treat diabetes in several parts of the world[10,11]. *Vinca rosa*, often known as *Catharanthus roseus* [10], is a herbaceous subshrub endemic to the Caribbean Basin that has been used to treat a variety of ailments in the past[12]. This plant's roots and leaves contain over 100 alkaloids. It is economically significant due to its alkaloids. Vinblastine and vincristine are the two most important leaf alkaloids in medicine, and they are used to treat cancer[14]. Ajmalicine (antihypertensive action) and serpentine (tranquillizer), indole alkaloids derived from the roots, are medicinally important indole alkaloids[13]. Vinca alkaloids are a class of medications derived from the periwinkle plant of Madagascar. They're made from the pink periwinkle plant(vinca rosa), and they have hypoglycemic and cytotoxic properties. They've been used to treat diabetes, high blood pressure, and disinfectants, among other things. Cancer-fighting properties of the vinca alkaloids are also essential. Vinblastine (VBL), vinorelbine (VRL), vincristine (VCR), and vindesine are the four main vinca alkaloids used in clinical practise (VDS). In the United States, VCR, VBL, and VRL have been approved for use. Vinflunine is a novel synthetic vinca alkaloid that has been licenced for the treatment of second-line transitional cell carcinoma of the urothelium in Europe and is being developed for additional
cancers.Vinca alkaloids are the second most common ly used family of cancer medications, and they will c ontinue to be among the first cancer treatments.
In this regard, various investigations and research for new vinca alkaloid applications will be conducted [15].

Antimicrotubule Vinca alkaloids, such as vinblastine and vincristine, interfere with microtubule dynamics and have been proven to induce apoptosis in a variety of tumour cells, resulting in cell death [16].

This research's major purpose was to determine the antioxidant and anticancer characteristics of two Egyptian plant extracts and their mode of action.

2. Experimental

2.1. Reagents and chemicals

All of the organic solvents used in this study were provided by Merck Chemical Inc (Darmstadt, Germany). Sigma Aldrich supplied Neutral Red [3-Amino-7-dimethylamino-2-methylphenazine hydrochloride] (St Louis, MO, USA). Gibco Inc. provided fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM)/high glucose, L-glutamine, and penicillin/streptomycin (NY, USA). The TC-treated 96-well clear flat-bottom polystyrene Microplates, 75cm2 U-Shaped canted neck cell culture flask with vent cap, Falcon tubes (15 and 50 ml) polystyrene centrifuge tubes, and sterile individually-wrapped Stripette TM serological polystyrene pipettes were all purchased in the United States. The Alex biotechnology firm provided all chemical reagents and buffers (Alexandria, Egypt).

2.2. Harvesting and preparing samples

The plants studied were obtained from the form of Faculty of Agriculture's, Cairo University, Egypt. They were recognized and certified by the Department of Crop Plants, Faculty of Agriculture, Cairo University, and Orman Garden in Giza, Egypt.

Healthy and fresh plant parts that had been washed and cleaned well were left for 3 days of air drying at ambient temperature. Finally, the air-dried parts were ground into a fine powder with a stand mixer and kept at 4°C in sealed containers until use.

Briefly, 100 grams of the dried samples were extracted using 80% ethanol (250 ml x 2 times) in a sonication bath at room temperature for 30 minutes then filtered and centrifuged at 6000g for 15 min. The supernatants were subjected to rotary evaporator to evaporate each extract at 45°C. Finally, the prepared extracts were saved at 4 ºC until use [21, 22]. For bioassays, dimethyl sulfoxide (DMSO) was used to prepare every extract's stock solution (1mg/ml) for complete solvation then tested for the anticancer assays or ethanol in antioxidants assays, respectively.

2.3. Determination of total phenol contents.

Polyphenolic compounds were determined using the Folin–Ciocalteu technique with slight modifications [13]. Ten µg of extract or gallic acid as a standard (0-200mg/l) was diluted with 2.8ml

ethanol (80%) and then mixed with 2 ml Na2CO3 (2%). 0.1 ml of 50% Folin–Ciocalteau solution was added and the mixtures were incubated at room temperature for 30 minutes. Finally, at 750 nm, the reaction mixture was read using spectrophotometer and compared to deionized water as a blank [23]. The total phenols were measured in triplicate, and the outcomes were measured in milligrams of Gallic acid equivalent per gram of dry weight (DW).

2.4. Determination of total flavonoids.

According to Chang et al., (2002 [24].), the flavonoid content of every extract was evaluated using the aluminum chloride colorimetric assay. 10 µg of each extract or a standard of Quercetin (0-50 mg/l) were diluted in 1.5 ml of 80% ethanol. After that, 0.1 mL of aluminum chloride hexahydrate (AlCl3) (10%) was added. Then 0.1 ml (1M) potassium acetate was added, followed by 2.8 ml deionized water. The mixture was then incubated for 40 minutes at room air temperature. Finally, the reaction mixture was read at 415 nm and compared to deionized water as blank. The total flavonoid content was measured three times and the results were represented in milligrams of quercetin equivalent per gram of dry matter.

2.5. Antioxidant activity

2.5.1. Radical scavenging activity of DPPH

DPPH assay was used to assess both extracts' free radical scavenging capability [25,26]. 1 ml of DPPH solution (0.1 mM) was mixed with 1 ml of plant extract at various concentrations or butylated hydroxyl toluene (BHT) as a standard. After that, the mixture was properly mixed before being incubated at room temperature in the dark for 30 minutes. The mixture was finally measured at 517 nm and the following equation was used to compute the % inhibition:

Antioxidant % = (A1–A2)/A1)×100

A1 represents the DPPH solution's absorbance in the absence of extract, and A2 represents the sample's absorptivity

2.5.2. Reducing power assay

In the presence of plant extracts, Fe3+ was reduced to Fe2+, suggesting the ability of the reductive extracts according to Aboul-Sound et al., [26]. 1.0 ml of deionized water containing various concentrations of the standard (Ascorbic acid) and plant extracts (400 to 6000 g/ml) was combined with 2.5 ml of phosphate buffer pH 6.6 and 2.5 ml of potassium ferricyanide 1 percent. For 20 minutes, at 50 degrees Celsius and the mixture was incubated. Then 2.5 ml of 10% trichloroacetic acid were added, and at 3000 rpm, the mixture was centrifuged for 10 minutes. The extravagant was added to 2.5 ml distilled water and 0.5 ml ferric chloride 0.1 percent reagent. At 700 nm, the absorbance was measured and the increase in absorbance of the reaction mixture was used to calculate the reducing power.

2.6. Anticancer activity

2.6.1. Cytotoxicity and cell viability

Human colorectal adenocarcinoma cells (Caco2) received from National Research Center and Ehrlich Ascites Carcinoma cells (EACC) from oncology institute Giza, Egypt. Two cell lines were used as a model to test both plant extracts. The cells were kept in flasks for tissue culture at 37°C and 5% CO2 in RPMI media containing fetal bovine serum (10%) and 100 g/ml penicillin/streptomycin. Trypsin was used to treat Caco2 cells, and trypan blue was used to determine cell count and viability using a hemocytometer. The cells were then seeded onto 96-well plates (2 ×103 cells/well in 150 µl of media) for 24 hours before being treated with 12.5, 25, 50, and 100 µl of plant extracts for 24 hours. [27, 28, 29,31].

2.6.2. Cell viability assay

After a 24-hour incubation period, 100 µl of Neutral red were added and incubated for 2 hours at 37 °C in the gloom. Then, to separate Neutral red dye from the cells, 150 µl neutral red destain solution were added. A microplate reader was used to measure the absorbance at 540nm. The viability (percent) was calculated when the specimens were evaluated in triplicate [30].

2.7. Gene expression profiling by RT-qPCR

Our hypothesis *Moringa oleifera* and *Vinca rosa* might contains specific phytochemical induces apoptosis, for that we tested that gene expression of key pro- and anti-apotosis markers in Caco2 cells exposed to IC50 [32]. In Brief, after 24h of treatment cells were collected and total RNA was extracted using Total RNA Purification Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. Then, cDNA synthesized using the QuantiTect Reverse Transcriptase Kit (Qiagen, Germany). The synthesized cDNA was subjected to QuantiTect SYBR-Green PCR Kit (Qiagen, Germany) as previously documented following standard protocols [32]. The primers

sequence used in this assay were showed in Table 1. Data were analyzed using the Rotor-Gene® cycler software 2.1 (Qiagen GmbH, Düsseldorf, Germany) to calculate the threshold cycle (Ct). The fold-change value was calculated after normalization to the expression of GAPDH gene, which was calculated using the equation $2^{-\Delta\Delta Ct}$.

Table 1. Primer sequence used in gene expression

Gene	Primers
Bax F Bax R	AAGCTGAGCGAGTGTCTCCGGCG CAGATGCCGGTTCAGGTACTCAGT
P53 F P53 R	GCTCTGACTGTACCACCATCC CTCTCGGAACATCTCGAAGCG
Bcl-2 F Bcl-2 R	CTCGTCGCTACCGTCGTGACTTGG CAGATGCCGGTTCAGGTACTCAGT
Casp-3 F Casp-3 R	AAACTTTTTCAGAGGGGATCG GCATACTGTTTCAGCATGGCA
GAPDH F GAPDH F	CGGAGTCAACGGATTTGGTC AGCCTTCTCCATGGTCGTGA

2.8. HPLC analysis

HPLC conditions

For HPLC analysis, the Agilent 1260 series was used. A C18 column (4.6 mm x 250 mm i.d., 5 m). The mobile phase consisted of water (A) and 0.02 percent tri-floro-acetic acid in acetonitrile at a flow rate of 1 ml/min (B). The following is how the mobile phase was designed in a linear gradient: 12-14 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14-16 min (80 percent A); 14 (80 percent A). The multi-wavelength detector was examined at 280 nm. The injection volume for each of the sample solutions was 10 l. The column was held at a constant temperature of 35 °C.

2.8. Statistical analysis

The data is provided as a mean with a standard deviation of three (n=3). The data were analysed using SPSS (version 20), and a p-value < 0.05 was used.

3. Results and discussion

3.1.Total phenolic(TP) and flavonoid(TF) contents

The Folin– Ciocalteu technique was used to figure out the total phenols in Moringa and Vinca ethanolic extracts. The table below presents the total phenolic content of the two plant extracts (Table 2). Total phenolics in vinca and Moringa extracts were 164.43 mg and 160.60 as gallic acid/g dry sample respectively. In agreement with the phenolic content measurements, total flavonoid content was 141.9 and 137.8 mg/g dry weight (as Quercetin equivalents). These data indicated that most of the phenolic contents are flavonoids. Therefore, these plants are enriched in their flavonoids contents. Different plants in our study contain high phenolic and flavonoid compounds, which potent antioxidant activity. The moringa and vinca ethanol extracts have high TPC and TFC.

Table 2. Total phenolic and flavonoid contents of Moringa and vinca extracts

Plant	Phenols GAE (mg.g-1dw)	Flavonoids QE (mg.g-1dw)
Moringa	161.60±0.488	137.8±0.66
Vinca	164.43±4.667	141.9±0.524

Results expressed as mg Gallic acid equivalent per 1mL of Ethanol extract. All values represented as mean ± SD (n=3); Different superscripts in the same column mean significant difference (P<0.05).

3.2. Antioxidant properties

3.2.1. radical scavenging activity using DPPH assay

Antioxidant scavenging potential was evaluated by using a DPPH assay. In this assay, serial quantities of each plant extract were utilized, as shown in (Table 2). As a result, Moringa extract showed the highest antioxidant activity than Vinca extract. The IC50 was calculated and indicated that Moringa extract has higher potential antioxidant activity than Vinca extract with IC50 55.35 and 185.05 µg/ml, respectively. The antioxidant activity was thought to be due to several phytochemical compounds, which were found such as catechin, chlorogenic acid, and propyl gallate which have the highest antioxidant activity [18].

3.2.2. Reducing power assay

The previous results showed that Moringa extract displayed higher antioxidants activity than vinca extract using DPPH assay, which is based on radical scavenging potency. The test for reducing power is based on the potential reduction, that reduced

potassium ferricyanide (Fe3+) is converted into potassium ferrocyanide (Fe2+). This is turning ferric chloride into a ferric–ferrous complex with a maximum absorption wavelength of 700 nm. The results showed similar effects for Moringa and Vinca extracts compared to standard ascorbic acid (Table 4). Data showed that the reducing power was slightly higher in Moringa extract than Vinca extract.

Table 3. Radical-Scavenging activity of moringa and vinca extracts using DPPH assay.

Plant extracts	% inhibition DPPH						
	Different concentrations (µg.ml^{-1})						
	10	50	100	200	400	800	1000
Moringa	40.32±0.7	47.15±1.85	57.7±0.31	81.11±2.6	85.92±0.86	91.72±0.32	96.74±0.31
Vinca	37.16±0.73	40.84±0.62	45.45±0.23	56.26±1.03	73.71±0.54	78.21±0.23	84.83±1.92

All values represented as mean ± SD (n=3)

Different superscripts in the same column mean significant difference (P<0.05)

Table 4.Reducing power activity of moringa and vinca extracts.

Plant extracts	Different concentrations (µg.ml^{-1})						
	400	600	800	1000	2000	4000	6000
Moringa	3.7±0.006	7.63±0.008	10.9±0.022	15.9±0.034	19.1±0.026	22.95±0.014	27.31±0.009
Vinca	3.09±0.002	5.45±0.004	8.24±0.003	9.22±0.004	20.18±0.011	25.3±0.013	28.18±0.027

All values represented as mean ± SD (n=3)

Different superscripts in the same column mean significant difference (P<0.05)

3.3. Anti-cancer activities

3.3.1. In vitro cytotoxic effect of Moringa and Vinca extract.

The cell viability was tested against caco2 and EACC after 24 h of treatment at various doses (12.5, 25, 50,100, 250, and 500 µg.ml-1) of each plant extract. The obtained results showed that Moringa extract displayed higher cytotoxic effects than Vinca extract against the caco2 human colon cancer cell line (Fig 1). The IC_{50} value of moringa and vinca was 71.34 and 83.73 µg.ml-1, respectively. Similar effects were observed against the EACC animal model cancer cell line (fig 2). The IC_{50} value of moringa and vinca against the EACC cell line was 381.12 and 593.34 µg.ml-1, respectively (Fig 2).

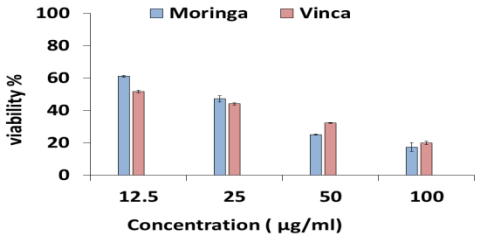

Figure1. Viability (%) of *Moringa oleifera* and *Vinca rosa* at different concentrations (µg/ml) of ethanolic 80% extracts and data represented mean ± SD (n=3) against caco2 cell line.

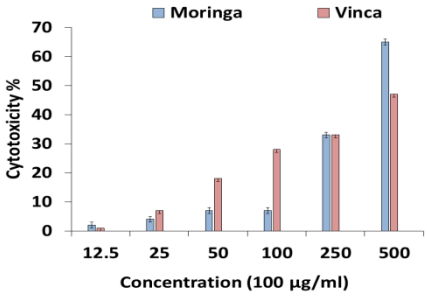

Figure 1. Cytotoxic activity (%) of *Moringa oleifera*and *Vinca rosa* at different concentrations (µg/ml) of ethanolic 80% extracts and data represented mean ± SD (n=3) against EACC animal model cancer cell line.

3.4. *Apoptotic gene expression.*

To determine the mode of action underlying the growth inhibitory effects Moringa and Vinca extracts against Caco2 cancerous cell line, the apoptosis induction was analysed. We tested the gene expression of various signal transduction key markers controlling apoptosis process, such as p53, Bax, caspase-3 and Bcl-2 key markers. The RTqPCR-results showed that, apoptosis-induced related genes p53, Bax and Caspase-3

were significantly up-regulated in Caco2 cells treated with the IC50 of each Moringa (Figure 3). Whereas, Bcl-2 gene expression was reduced by 0.4 fold change compared to untreated cells. The same pattern was observed after Vinca extract treating Caco2 cells with the IC50 doses. However, Moringa extract was more effective compared to Vinca extract. In general. Moringa have potential apoptosis induction compared to respective control.

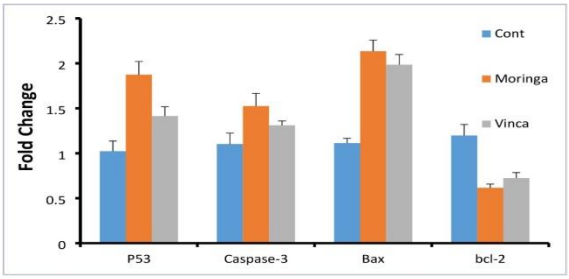

Figure 3. Fold change value of P53,Caspase-3, Bax and bcl-2 apoptotic genes treated by *Moringa oleifera* and *Vinca rosa* extracts.

3.5. *Identification of phenolic compounds in Moringa and Vinca extracts*.

Based on the obtained results by Moringa and vinca extracts as antioxidants and anticancer potencies, Following that, the fractions were subjected to a full-scan procedure, HPLC analysis to identify phenolic compounds against the most abundant standard phenolic compounds present (Tables 5). These plant extracts contained phenolic substances such as phenolic derivatives (catechin, vanillin, syringic acid, catechol) and flavonoid glycosides such as quercetin and isoquercitrin rutin. In Moringa extract, the most abundant phenolic components were Chlorogenic acid 244.18 g/ml, Catechin 196.71 g/ml, and Rutin 149.70 g/ml, according to peak areas. Vinca extract Catechin was the predominant phenolic compound with 341.69 µg/ml followed by Propyl Gallate 188.65 µg/ml.

These results may explain the high potency of these compounds as antioxidant and anticancer activities. In this concern, the mode of action of these compounds need more work and deep explanation.

Table 5. HPLC analysis of phenolic compounds in moringa and vinca extract

Phenolic compound	*Moringa* EX Conc.(µg/ml)	*Vinca* EX Conc.(µg/ml)
Gallic acid	ND	5.39
Chlorogenic acid	244.18	44.81
Catechin	196.71	341.69
Coffeic acid	12.81	12.5
Syringic acid	13.98	20.02
Rutin	149.7	53.49
Ellagic acid	ND	44.13
Coumaric acid	3.41	37.2
Vanillin	47.08	7.36
Ferulic acid	7.5	30.98
Naringenin	33.14	45.44
Propyl Gallate	25.76	188.65
Querectin	42.68	27.81

All results expressed as µg/ml of dry weight
ND: Not detectable.

4. Conclusions

According to the current study, plant extracts offer numerous active qualities, including antioxidant, anti-cancer and apoposis potential. Moringa and Vinca ethanol extract with high phenolic content efficiently reduced in vitro free radical activity, implying that these medicinal plants have a unique natural antioxidant role. Furthermore, the results revealed that Moringa extract had a stronger cytotoxic effect on the caco2 human colon cancer cell line than Vinca extract. Moreover, similar products were reported against the EACC cancer cell line from an animal model. Moreover, Moringa have potential apoptosis induction compared to respective control. As a result, these plants had several phenolic and fhavonoid compound which are mainly responsible for these activities. We recommend these plants to be used as a good natural source for biological activities as antioxidant, gene apoptosis and anti-carcinogenic potential.

5. Interest Conflicts

There are no conflicts of interest declared by the authors. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript writing, or the decision to publish the outcomes.

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