Phytochemical Analysis, Antioxidant and Cytotoxic Potential of Rumex Vesicarius Extracts

Student Sabreen A. Alazzam[1], Mohammed Mosleh Sharqi[2], Ali F. AlMehemdi[3]

[1]Department of Biology, College of Science, [2]Lecture, [3]Prof., Center of Desert Studies, University of Anbar, Iraq

Abstract

Background: *Rumex vesicarius L.* (Polygonaceae), an edible plant, is documented to have many bioactive phytochemicals. In spite of it fully -known antioxidant efficacy of crude extract Rumex vesicarius but still has the cytotoxicity.

Methodology: Methanolic extraction was prepared from leaves of Rumex vesicarius and was checked by GC/MS. Two parts of assay were classified as : First, evaluation antioxidant potency using DPPH free radicals, while the two assay of MTT was utilized to determine anti-cancer effect on two different cell lines (MCF-7 and WRL68) for different concentrations (400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml and 25µg/ml and 6.2 µg/mL) and identified the efficacy of the crude extract on MCF-7 cell morphology, response to stress, potential of mitochondria and viability were evaluated with High Content Scanning and MTT.

Results: GC/MS analysis showed the methanol extract of Rumex Vesicarius L. had the highest percentage of L- Pidolic Acid (35.98%), vitamin E (12.05%). *R. Vesicarius* showed antioxidant activity which increased when increasing of concentration, *R. Vesicarius* effect ranged from (75.935,9.96%). The results showed *Rumex vesicarius* ability to inhibit cellular growth of both cell lines (WRL68 and MCF-7). This inhibition increases with increasing concentration. The best inhibition of growth was at 400µg/ml of Methanol extract. The MCF-7 cell line was more effective than the WRL68 cell line, High Content Screening (HCS) assay of 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml of methanol extract of R. Vesicarius showed toxic effects toward MCF-7 cell line after 24 hours of treatment in a dose-dependent manner with a reduction in the number of total cell count, reduction in the mitochondrial membrane potential (MMP), an increase in the nuclear intensity, increase in the membrane potential and increase in cytochrome C.

Conclusion: Methanolic extract of *Rumex vesicarius* contains many bioactive phytochemicals and appear to have cytotoxic and antioxidant activity against MCF-7 & WRL68 Cell line.

Keywords: *Rumex vesicarius, Medicinal plants, Cytotoxicity, GC-MS analysis, High content screening.*

Introduction

Rumex vesicarius is an annual plant, which belongs to family Polygonaceae, commonly known as "Bladder dock or Chukkakura or Khatta Palak"[(1)]. Rumex vesicarius L is widely utilized as a medicinal herb or as food[(2)]. It is used in the treatment of liver defect, cancer, cardiovascular diseases and cataract [(3)], digestive problems, toothache, nausea, pain, anti-inflammatory, antitumor as well as antischistosomal, and antimicrobial activities[(4)]. It was also found to have an aphrodisiac effect[(5)]. Previous chemical investigations have shown the presence of polyphenols, flavonoids, carotenoids, tocopherols and ascorbic acid in different organs extract from *Rumex vesicarius L.*[(6)], Polyphenols have essential roles such as functioning as antioxidant, anti-inflammatory, anticancer agents antimicrobial and antiallergic[(7)].

Phenolic compounds, tannins, anthocyanin, and flavonoids can play a role in free radical scavenging inhibition through different mechanisms[(8)]. Plant leaves

are rich in ascorbic acid, citric acid and tartaric acid; it also contains glycoside, alkaloid, flavonoids, tannins and phenolic compounds[9].

The aims of this study were to judge the antitumor and inhibitor activity of R. vesicarius crude extracts in vitro used the human breast glandular cancer MCF-7and WRL68 cell line via has known of inhibitor activities against DPPH and evaluated the anti-tumour activity by using MTT assays and HCS for detective work the subsequent cellular parameters: membrane porousness, Cell viability, total nuclear intensity, cytochrome unleash and mitochondrial membrane potential changes.

Materials and Method

Materials: All artificial substances were luckily given from Al-Nahrain University, Department of Biotechnology. The MTT unit was nonheritable from desoxyribonucleic acid Biotechn, whereas 1Cellomics®1 Multiparameter of toxic three pack were obtained from ThermoScientifick (America).

Plant Materials: Leaves part of R. vesicarius were gathered from Ramadi University of Anbar, Iraq.

Methanolic Extraction: The leaves *of R. vesicarius* was dried and convert to powder form as 0.1 g then diluted with 400 mL alcohol methyl. Then homogenate the mixture using of a hot plate stirrer for four h at 37°C and then filtrated. The solvent was centrifugated with 1600 rpm at 10°C for 20 min. The alcoholiccrude material was dried at 46°C using a rotary evaporator) Germany) under vacuum to get the dried extract.

GC/MS Analysis: The extract of R. vesicarius was analysis by[GC-2011 with equipped with DB-5MS column (30 m long zero.25 mm i.d. and 0.25 um thick, Agilent Technologies, J and W Scientific product, America)]. The temperature of gizmo and detector were set at 240 and 235°C, severally. The kitchen appliance temperature began with 100°C and raised till reach to 261°C for sixty sec. One µL of the sample as an aliquot of was injected, and gases noble gas was used.The mass varies scanned from[(50-550) amu. Identification of matter and essential oils was done by the Ministry of Science and Technology, Department of Water and Environmental analysis (using government agency Library)].

Evaluation of antioxidant activity using DPPH: activity of inhibitor was calculable utilizing DPPH as radical commonplace looking on an antecedent study[10].

Cell culture: Adenocarcinoma of breast tissue MCF-7 cells [11]. And Hepatic tissue WRL68 Cell Lines [12] were purchased from USA Type Collection Cultured.

MTT cytotoxicity assay: The assay was done according to the instructions of the company, this test done according to [13].

Multi-parameter cytotoxic assay: The double-parameter toxicity experiment was done on live the 5 orthogonal MCF-7 cells normally health index once received to R. vesicarius crude alcoholic extract in vitro. The parameters were: Viability cell count, total nuclear intensity, semi-permeable membrane porousiness, mitochondrial membrane porousness and cytochrome unharness. Briefly, once twenty-four h of exposure with totally different concentrations of R. vesicarius alcoholic crude extract, the treated MCF-7 cells were stained with cell staining resolution for thirty min at 37°C. Cells were mounted, permeabilized and blocked before inquiring with primary cytochrome protein and secondary DyLight [649 conjugated goat anti-mouselgG for sixty min] every. Plates were analyzed victimisation the ArrayScan HCS instrument.

Statistical data: A unidirectional analysis of variance (ANOVA) was done to evaluate whether or not cluster different was vital. Knowledge was represent as (Mean ± standard error) (SE), and applied mathematics significances were allotted exploitation a Graph Pad Prism version.

Results

The analysis of GC/MS: methanol extract of *R. vesicarius* was showed in Table 1 and Fig 1, it appeared that the crud extract involves 24 active ingredients which represent 93.02%. The are many active ingredient was Pidolic Acid 35.98% followed by Phthalic acid, diisooctyl ester 12.09%, Vitamin E (α-Tocopherol) 12.05, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- 10.41%, 13-Tetradece-11-yn-1-ol 9.01%, n-Hexadecanoic acid 5.54%, γ-Tocopherol 4.75%, Formic acid, 1-methylethyl ester 3.59%, Hexadecanoic acid, 15-methyl-, methyl ester 2.87%, α -Tocopherol 2.67%, 2-Methoxy-4-vinylpheno 11.09%. In addition, 10 parts were present in an amount less than 1%.On the other aspect, 9.97% still non-identified constituent.

Table 1: Chemical contents of R. vesicarius alcoholic extract.

Peak	Rt (min)	Compound	Area %
1	2.41	Formic acid, 1-methylethyl ester	3.59
2	11.45	2-Methoxy-4-vinylphenol	1.09
3	12.48	Pidolic Acid - L	35.98
4	18.92	Hexadecanoic acid, 15-methyl-, methyl ester	2.87
5	19.34	n-Hexadecanoic acid	5.54
6	20.73	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	10.41
7	21.15	13-Tetradece-11-yn-1-ol	9.01
8	24.70	Phthalic acid, diisooctyl ester	12.09
9	28.12	Δ-Tocopherol	2.67
10	29.10	γ-Tocopherol	4.75
11	30.30	Vitamin E (α-Tocopherol)	12.05

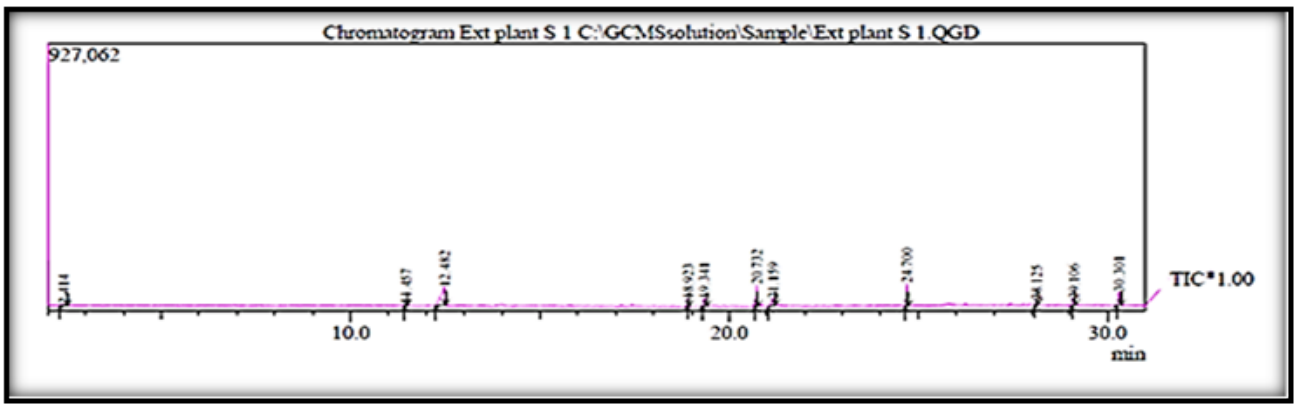


Fig. 1: GC-MS chromatogram of Rumex vesicarius L. of methanolic extract.

Antioxidant activity of Rumex vesicarius **crude extract:** The DPPH scavenging effect of *R. vesicarius* methanol was estimated. Data showed that with elevating dose of extract, the scavenger percent activity was increase. The doses of plant that were utilized from 12.5-100µg ml^{-1} and the plant appear altitude antioxidant activity to give significantly value (75.63% at 100µg ml^{-1}). The data in Table 1, when compared with ascorbic acid standard drug the free radical scavenging activity, showed percent near from free radical scavenging activity, especially at high dose of extract with 50 and 100µg ml^{-1}.

Cytotoxic result of R. vesicarius extracts on MCF-7cells and WRL68 Cell Lines mistreatment the MTT assay: Our result results showed that R. vesicarius extract has poisonous effect on carcinoma cells in dose dependent manner (Table 2).

Table 2: Anticancer activity of methanolic extract of R. vesicarius leaves on MCF-7 and WRL68 cell lines by using MTT method after 24 h. of incubation at 37°C.

IC50	6.2	12.5	25	50	100	200	400	Concentration (µg/ml^{-1})	
111.9	95.95±0.5	95.72±1.2	95.18±0.8	78.74±7.0	68.79±4.0	46.10±4.6	36.36±3.0	MCF7	Methanol extract
~ 12075	98.65±0.2	97.15±4.0	96.49±2.5	95.95±2.6	93.60±2.1	90.59±1.4	78.13±3.5	WRL68	

*MCF-7: human Breast cancer cell line, WRL68: human hepatic cell line.

Multi-parameter cytotoxic activity of R. vesicarius extract : Table (3) showed that 200 µg/ml of methanolic extract of *R. vesicarius* has cytotoxicity effect on MCF-7 cell line. 50 µg/ml, 25 µg/ml and 12.5 µg/ml Showed results close to those of the untreated cells which represent the negative control with a very few significant differences.

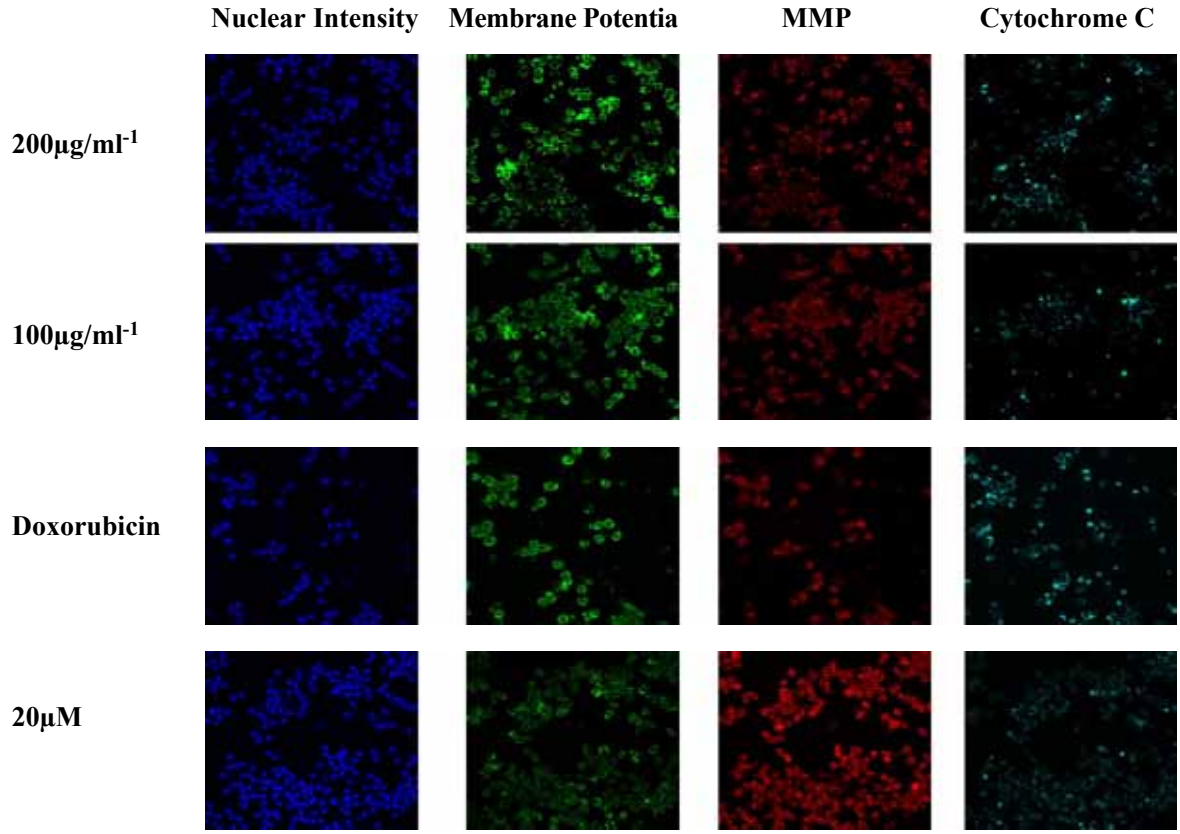
From (Table 3 and Fig 2) methanolic extract significantly increased the nuclear intensity of the MCF-7 cell line. This increasing was dose dependent (37.8 %, 8.2%, 7.3%, 2.8%, and 6.8% for 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml respectively). The highest percentage of increasing was 37.8% at 200µg/ml when compared with untreated. 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml did not show any significant differences from untreated.

Results of cytochrome C releasing listed in (Table 3) rise significantly with the increasing of concentration when compared with untreated and the percentages of increasing were 34.9%, 12.7%, 1.0%, 2.9% and 11.2% for 200 µg/ml and 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml respectively.

Table 3: Cytotoxicity effect of methanolic extract of R. vesicarius on multi cellular parameters after one day of overnight at 37 º C.

Concentration ($\mu g/ml^{-1}$)	HCS Parameters (Mean±SD)									
	Valid Cell Count	IH*	NI**	IH*	MMP***	IH*	MP****	IH*	Cytochrome C	IH*
Dox 20	1411±90.6	58.6	878.5±72.83	101.7	245.0±26.87	307.5	250.3±26.87	87.3	732.0±166.3	73.2
Untreated	3411±163.4	0.00	435.5±34.65	0.00	552.5±96.87	0.00	133.6±19.10	0.00	422.5±87.8	0.00
200	2923±454.1	14.3	600.5±14.85	37.8	361.0±50.21	34.6	156.5±4.96	17.1	570.0±75.0	34.9
100	3238±226.4	5.0	471.5±33.23	8.2	426.5±45.70	22.8	151.5±20.52	13.3	476.5±173.6	12.7
50	3451±24.5	1.1	467.5±38.89	7.3	468.0±21.93	18.0	141.1±9.91	5.6	418.5±94.4	1.0
25	3441±186.8	1.0	423.0±50.92	2.8	505.0±21.93	8.5	140.1±11.32	4.8	410.0±37.9	2.9
12.5	3284±62.3	3.8	405.5±24.75	6.8	537.0±35.36	2.8	132.6±19.10	0.7	375.0±7.3	11.2

*In Hibition,**Nuclear Intensity, ***Mitochondrial Membrane Potential, **** Membrane Potential



Vehicle Control: Fig.2: The analysis of *R. vesicarius* extract by HCS) after treated the (MCF-7) cell line after one day of incubation at 37ºC. The stained cell by Hoechst 33342 (Blue)(Ex330nm/Em420nm) dye which capable of monitoring of cell loss, permeability dye nuclear morphology changes, (Green) (Ex491nm/ Em509nm) for monitor the membrane permeability, MMP dye (red)(Ex552nm/Em576nm) for changes potential mitochondrial membrane (PMM) and with second antibody conjugated with DyLight TM for cytochrome C releasing.

Discussion

In the current report, analysis by GC-MS of the *R. vesicarius* extract of a plant of leaves showed the presence of 11 compounds. Pidolic Acid, Phthalic acid, diisooctyl ester, Vitamin E (α-Tocopherol), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, 13-Tetradece-11-yn-1-ol, n-Hexadecanoic acid, γ-Tocopherol, Formic acid, 1-methylethyl ester, Δ-Tocopherol and 2-Methoxy-4-vinylphenol were consistent in the plant. These essential agent have all appear to have cancer inhibition, nematicide, pesticide, lubricant, antiandrogenic, insectifuge, 5-Alpha reductase inhibitor activity, antioxidant, hypochloesterolemic, haemolytic. There is a growing awareness in correlating the phytochemical compounds and their biological activities [(14)].

Our study report the confirm of some of the essential agent identified by GC-MS analysis and their biological potency. Thus, this type of GC-MS analysis is the first line towards check the nature of active principles in this natural extract and this type of study will aid for further detailed in the future study.

Number of plants extract possess activity of antioxidant, which can limit the ROS in tissue and thus aid in a cure for different human problems, involves neoplasm, cardiovascular defect and inflammation [(15)]. The *R. vesicarius* crude extract is often utilized as a medicinal herb due to biological activities,thus antioxidant activities [(16)]. However, little report about the effect of extract on antitumor activity. The harmful effect recorded with MCF-7 cells received to alcoholic crude extract maybe because of the contents of bioactive compounds, where they believe to be the major active ingredients of *R. vesicarius* that lead to a huge variety of biological effects. Such results are in concord with[(17)], which reported results showed that all extracts possessed concentration-dependent antioxidant activity. In addition, this study concludes that *Rumex vesicarius L* possesses diverse therapeutic potentials that might be used as natural antioxidant and antibacterial [(16)]. Some studies demonstrate that all the extract of *Rumex vesicarius*. Shows the significant immunomodulatory effect on both humoral as well as cell-mediated immunity [(18; 19)]. On the other hand, the use of dye stained the membrane permeability and the elevated of intensity of this stains, especially at the massive dose received, potentiate the fact that the crude extract can stimulate apoptosis in MCF-7 cells, the permeability of plasma membrane increases may be because the loss integrity of membrane that dye penetrate cell easily [(20; 21)].

The permeableness transition of mitochondrial pores, permitting the transition tiny molecules and ions, like atomic number 20 ions and therefore resulting in the decoupling of the metabolic process chain and unharness of cytochrome of the cytoplasm[(22)]. Finally, the secrete of a cytochrome stimulate a variety of caspases, specifically amino acid proteases, that ar essentially support digestion of the cell from within in addition as degradation[(23)].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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