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Traganum nudatum Possesses Anticancer Efficacy against Breast Cancer MDA-MB-231 Cells

Ibrahim O. Barnawi[1*]

[1]Department of Biology, Faculty of Science, Taibah University, Almadinah Almunawarah, 41321, Saudi Arabia.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Background: Medicinal plants are effective sources for both traditional and modern medicines; herbal medicine has been shown to have genuine utility. 80% of rural population depends on natural products as primary health care. *Traganum nudatum* (*T. nudatum*) is a medicinal plant widely used in alternative medicine. *T. nudatum* has not been greatly investigated for its anticancer potential.
Aim: This study aimed to evaluate the *in vitro* anticancer efficacy of *T. nudatum* against breast cancer cell line; MDA-MB-231.
Materials and Methods: Extracts of *T. nudatum* were evaluated to assess their *in vitro* cytotoxicity against the MDA-MB-231 breast cancer cell line. Leaves, flowers, and stems of *T. nudatum* were extracted by using 70 % ethanol. the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays were used to evaluate the *in vitro* cytotoxicity of these extracts against MDA-MB-231 cell line. The apoptotic cellular morphological changes were investigated using inverted and fluorescence microscopes.
Results: The results showed that *T. nudatum* extracts exhibited anticancer activity with a half-maximal inhibitory concentration (IC_{50}) of 150 µg/mL against MDA-MB-231 cell line. Loss of cell integrity, shrinkage of the cytoplasm, and cell detachment were observed in the extract-treated MDA-MB-231 cells.
Conclusion: *T. nudatum* extracts showed cytotoxic effects against MDA-MB-231 cell line. It may be used as a future medication to cure breast cancer.

**Corresponding author: E-mail: avcc20@gmail.com, Avcc300@gmail.com;*

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1. INTRODUCTION

Cancer is the second cause of death worldwide. In women, breast, lung, and colorectal are the most common cancer types, they account for an approximate 50% of all new cancer diagnosed in women in 2020. Worldwide, 19.3 million new cancer cases and 9.6 million deaths were estimated in 2020 [1]. Particularly, in Saudi Arabia, 17,522 cases (8,296 males and 9,226 females) were diagnosed as cancer patients, and the cancer death rate was 9,134 across all ages and gender [2]. The rate of cancer death is higher in female than male, it ranges from 0.1–9.1 in females and 0.1–7.3 in males [3]. The Saudi Cancer Registry (SCR) reported that breast cancer as the first encountered cancer among Saudis of all ages (15%) and ranked it first in newly diagnosed female patients (27.4%) [4]. For cancer treatment, surgical removal, chemotherapy, immunotherapy, gene-therapy, and nano-therapy were applied in preclinical and clinical settings. Even though, the conventional chemotherapy still the best and potential approach for cancer therapy, upon the use of chemotherapy, several side effects were reported [5]. To overcome side effects and chemo-resistance post chemotherapy treatments, different protocols using new natural anticancer agents are in need [6-8].

Phytochemical examination of several medicinal and wild plants was reported as of possible anticancer compounds. Today, an estimate 60% of drugs used for cancer treatment were produced from natural products [9]. Approximately, 60% of anticancer chemotherapeutic agents were originally isolated from natural sources such as plants and animals resources [9,10].

Traganum nudatum (*T. nudatum*) is a plant belonging to *Chenopodiaceae* family, which is a small shrub whose flowering takes place in the autumn. *T. nudatum* is a native halophytic shrub in arid zones of the Mediterranean basin, it grows at high temperatures and can tolerate increased salinity and aridity [11]. Moreover, *T. nudatum* can be used in traditional therapy to treat diabetes, diarrhea, rheumatism, otitis, skin diseases, and wound healing due to its antioxidant potentials [12-14]. In addition, it shows high antimicrobial activity against many species of bacteria and fungi. For instance, its extracts possess promising antibacterial activities against *Bacillus subtilis, Staphylococcus aureus, Enterococcus fæcalis, Listeria monocytogenes, Proteus mirabilis* [15]. The aim of this study was to investigate the effects of different *T. nudatum* extracts on cell proliferation, migration, and apoptosis in MDA-MB-231 breast cancer cell line.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh aerial parts of *T. nudatum* were collected and identified from Al-Madinah Al-Munawara, Saudi Arabia. At room temperature, the plant was left to air dry partially for 2-3 days. Then the plant was chopped and grounded by using an electrical grinder until it obtained powder. Each 50g of *T. nudatum* was extracted by an ethanol-water mixture (70/30 V/V) for 48 h. Then this step was repeated three times. After that, the filtrate was refluxed in (2L) 70 % ethanol at 50 °C using a rotary evaporator for 36 h in a continuous extraction (Soxhlet) apparatus (S.P. Verma - Popular Science Apparatus Workshops PVT LTD- India). Pooled and concentrated ethanol extract was filtered and re-concentrated under reduced vacuum pressure, keeping a constant temperature of less than 50 °C. The extract yield was kept at room temperature for later use.

2.2 Assessment of Cell Viability

Cell viability was assessed by an MTT assay [16]. Briefly, MDA-MB-231 and HepG-2 cells were seeded at 5×10^4 cells/well in 24-well plates and left to attach overnight. Different *T. nudatum extract* was applied on cancer cell lines and incubated for 48 h in CO_2 incubator. Then, 100 µL MTT solution (5 mg/mL in saline) was added to each well and incubated for 2 h at 37 °C. The culture media were aspirated and then 1mL methanol was added to dissolve the formazan crystals. The optical density (O.D.) value was read at 490 nm using a microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). The cell viability (%) was calculated using the following formula: The relative cell viability (%) = treated group/control group × 100%.

The half-maximal inhibitory concentration (IC_{50}) was calculated using origin software. Data were

represented as the averages of three independent experiments.

2.3 Lactate Dehydrogenase (LDH) Assay

The cytotoxicity of the extract was also assessed by the lactate dehydrogenase (LDH) release assay on MDA-MB-231 cells [17]. Briefly, MDA-MB-231 cell lines were treated with the different extracts for 48 h. Then, 100 µL of the culture medium was collected and 100 µL of LDH reaction solution (Sigma LDH Cytotoxicity Assay Kit, USA) was mixed for 30 min. The developed color was measured at 490 nm using a microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.4 Light and Fluorescent Microscopic Investigations

MDA-MB-231 cells were grown in 12-well plates and treated with the crude extracts at their IC_{50} for 48 h. The morphological changes were examined under an inverted light microscope attached to a Leica MC-170 HD camera (Leica, Germany). For Hoechst 33258 fluorescence staining, the cells were incubated with the extracts, washed twice with phosphate-buffered saline (PBS) at 20 °C, fixed with 4% paraformaldehyde, permeabilized with cold methanol, and then stained with 10 mg/mL Hoechst 33258 (Sigma-Aldrich diluted in PBS (final concentration 0.1 µg/mL) [18]. Then, the cells were observed for nuclear changes (chromatin condensation and nuclear fragmentation) and characteristics of apoptosis under a fluorescence microscope attached to an Axiocam 506 color camera (Zeiss, Germany).

2.5 Migration Assay

MDA-MB-231 cells (5×10^4) of were separately seeded per well in 6-well plates in DMEM medium supplemented with 10% serum, until confluence in a 5% humidified CO_2 incubator at 37 °C. A wound was created with a 200 µL pipette tip and the debris was removed by a wash with PBS. The cells were incubated with or without extract at the concentration of 40 µg/mL. The results were the average of three independent experiments. The cells were photographed using a phase contrast microscope (Leica, Germany) at three time points (0, 24 and 48 hours). The area of migration was measured using Image J software and the percentage of wounds recovered was calculated.

2.6 Annexin-V and Dead Cell Assay

The assay was determined using Muse™ Annexin-V & Dead Cell (7-AAD) kit (Merck Millipore, USA) as per the instructions of the manufacturer. Briefly, 1×10^5 cells/ well/1 mL culture media were seeded in a 6-well plate and incubated at 37 °C for 24 h. The cells were then treated with *T. nudatum* extracts for 24 h. The concentrations of the extracts used for MDA-MB231 were 250 µg/mL, and 500 µg/mL, respectively. After treatment with *T. nudatum* extracts, the cells were re-suspended in 1% FBS. One hundred microliter of the cells were transferred to 1 mL micro-centrifuge tubes and 100 µL of Muse™ Annexin-V & Dead Cell reagent was added and mixed with the cells, followed by 20 min incubation at room temperature in the dark. The cells were then analyzed using Muse™ cell analyzer. The assay could identify four types of cells: i) non-apoptotic live cells: Annexin-V (−) and 7-AAD (−), ii) early apoptotic cells: Annexin-V (+) and 7-AAD (−), iii) late apoptotic cells: Annexin-V (+) and 7-AAD (+), and iv) nonapoptotic dead cells: Annexin-V (−) and 7-AAD (+).

2.7 Statistical Analysis

The criterion for statistical significance was set at $p < 0.05$ or $p < 0.01$. All data are presented as mean ± SD. T test was used to compare all groups against the control group to show the significant effect of treatment.

3. RESULTS

3.1 IC_{50} of *T. nudatum* Extract against HepG-2 and MDA-MB-231 Cell Lines Post 48 h.

Different concentrations of *T. nudatum* extract ranged from 0 to 500 µg/ml were used to determine the IC_{50} against HepG-2 and MDA-MB-231 cell lines *in vitro* post 48 h of treatment. The results showed that the IC_{50} of *T. nudatum* extract against HepG-2 was 200 µg/ml and against MDA-MB-231 cell lines was 150 µg/ml (Fig. 1).

3.2 Treatment with *T. nudatum* Extract Increased LDH relEase from HepG-2 and MDA-MB-231 Cells *in vitro*

HepG-2 and MDA-MB-231 cell lines were incubated with *T. nudatum* extract to determine

its cytotoxic effect through releasing LDH *in vitro*. The data showed that compared to the untreated HepG-2 cells, the treatment with *T. nudatum* extract showed significant increase in the release of LDH post 48 h *in vitro*. Treatment of MDA-MB-231 cells with *T. nudatum* extract also showed significant increase in the LDH release post 48 h *in vitro* (Fig. 2).

3.3 Treatment with *T. nudatum* Extract Decrease the Migration Distance Rate of MDA-MB-231 cells *in vitro*

Effects of *T. nudatum* extract on MDA-MB-231 cells migration was determined. The cells were untreated (control) or treated with IC_{25}. MDA-MB-231 cell line was left untreated or treated with IC_{25} of *T. nudatum* extract. Images of the wounded monolayer of MDA-MB-231 cells captured immediately after wounding (t0 h) and during an incubation time of up 48 h. The results showed that exposure of MDA-MB-231 cells to IC_{25} of *T. nudatum* extract led to a significant decrease in the migration distance ration of MDA-MB-231 cells post 24 and 48 hrs of treatments. (Fig. 3 A and B).

3.4 Treatment with *T. nudatum* Extract Increase the Necrotic and Apoptotic of MDA-MB-231 Cells *in vitro*.

The percentages of the necrotic and apoptotic MDA-MB-231 cells (early and late apoptosis) were determined post 48 h of treatment with *T. nudatum* extract *in vitro*. As shown in Fig. 4 A and B, treatment with 250 µg/ml of *T. nudatum* extract increase the percentages of necrotic and apoptotic cells were 2.3 and 11.72%, versus 0.8 and 5.8% in untreated MDA-MB-231 cells. Treatment with 500 µg/ml with plant extract increased the above-mentioned values into 51.2% (necrotic cells), and 14.5% (total apoptotic). The highest ratio of dead cells and the lowest ratio of live cells were reported post treatment with 500 µg/ml for 48 h (Fig. 4 A and B).

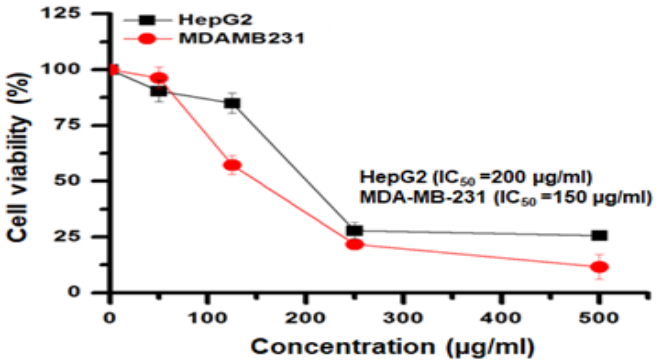

Fig. 1. The half-maximal inhibitory concentration (IC_{50}) of *T. nudatum* extract on HepG-2 and MDA-MB-231 cell lines post 48 h. Values were expressed as mean ± SD

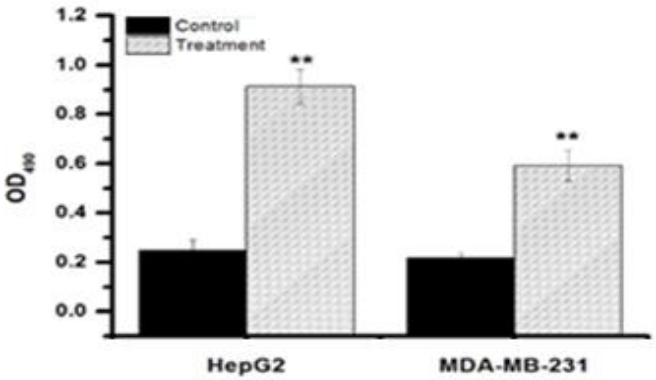

Fig. 2. Lactate dehydrogenase (LDH) release from HepG2 and MDA-MB-231 cell lines treated with *T. nudatum* extract for 48 h. *in vitro*. Values are expressed as mean ± SD

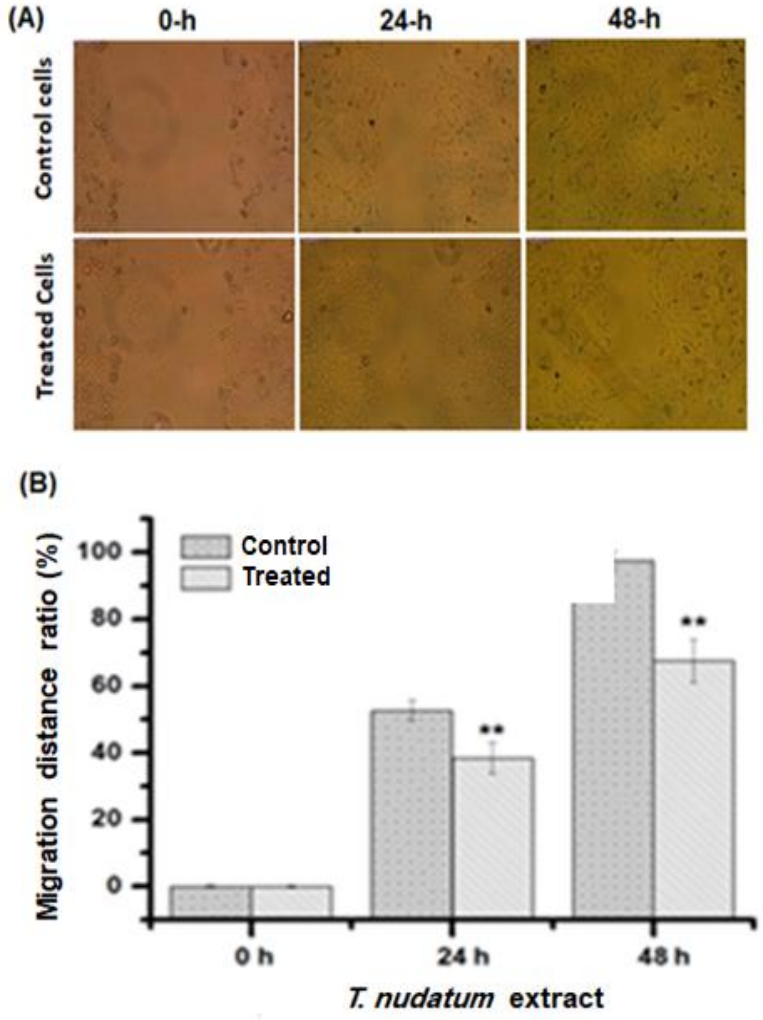

Fig. 3. Effects of *T. nudatum* extract on MDA-MB-231 cells migration. (A) Images of the wounded monolayer of MDA-MB-231 cells captured immediately after wounding (t=0 h) and during an incubation time of up 48 h. The cells were untreated (control) or treated with IC_{25}. (B) Cell migration rate calculated as described in materials and method. Values are expressed as mean ± std. (n = 3). The experiments were performed in duplicate, *P <0.05 vs. the control (Student's two-tailed t-test)

4. DISCUSSION

To find new anticancer agents safe and with little side effects on vital organs when compared to the current chemotherapeutic agents upon the treatments, several studies have been reported to screen and evaluate the anticancer efficacy of thousands natural components extracted, isolated, and purified from several medicinal plants [19]. The current study evaluated the anticancer efficacy of *T. nudatum* extract against MDA-MB-231 cell line *in vitro*. The study reported that the *T. nudatum* extract showed a moderate anticancer effect against MDA-MB-231 cell line *in vitro*. The IC_{50} of *T. nudatum* extract against HepG-2 and MDA-MB-231 cancer cell lines were 200 and 150 µg/ml, respectively. The anticancer effect of this extract could be due to the presence of potential phytochemicals constitute including alkaloids, saponins, tannins, flavonoids and quinones and the absence of triterpenes, anthraquinones, sterols and cardinolides [20]. This finding agrees with previous report showed that there is a significant positive correlation between various secondary metabolites content such as phenolics, flavonoids, saponins and anthocyanins with the total antioxidant capacity. These findings agreed with previous study reported the anticarcinogenic activity of plants extracts against tumor cell lines [21].

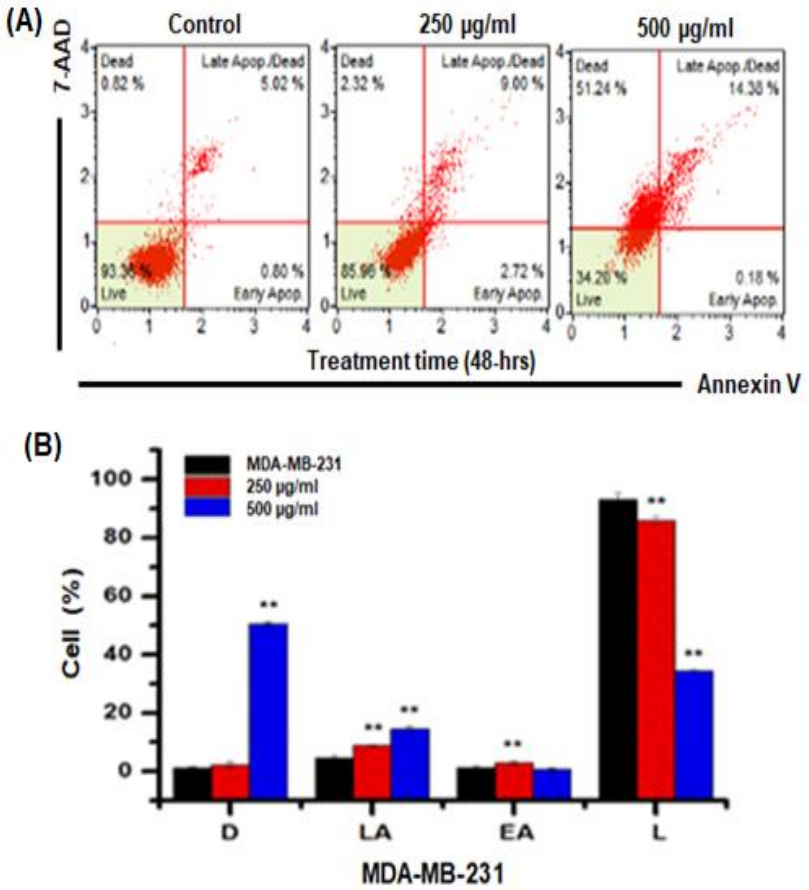

Fig. 4. Effect of the *T. nudatum* extract on the MDA-MB-231 cells. Representative Apoptosis profile of the treated and untreated cells after 48 h exposure to 250 or 500 µg/mL of the extract (a). The percentage of live (L), early apoptotic (EA) and late apoptotic (LA), and dead (D) cell populations of untreated and treated groups. Data represents mean ± SD from three independent experiments ($n = 3$). ** $p \leq 0.05$, significantly different from control

The LDH assay is a simple and fast cytotoxicity assay that measures the LDH released into the extracellular medium following cell membrane damage. The intracellular LDH released into the medium is a measure of irreversible cell death due to cell membrane damage, whereas Xia et al. (2007) reported the direct involvement of LDH upregulation and subsequent induction of apoptosis [22]. The cytotoxic effect of *T. nudatum* extract was further investigated by LDH assay, the results showed that the extract had cytotoxic effect on HepG-2 and MDA-MB-231 cell lines *in vitro* evidenced by increasing the release of LDH post 48 h of treatment.

The present study reported that the treatment of MDA-MB-231 cell line *in vitro* with IC_{25} of *T. nudatum* extract decreased the migration distance ratio when compared to the control untreated cancer cells. The morphological changes in the apoptotic cells were also apparent in the Hoechst 33258 staining, which facilitated the detection of cell death caused by apoptosis. Induction of apoptosis has been considered as a hallmark for the identification of anticancer drugs [23]. One of the noteworthy findings of this study was that *T. nudatum* plants induced apoptosis. Exposure of MDA-MB-231 cell line to 250 or 500 µg/ml of *T. nudatum* extract for 48 h. increased the percentages of the necrotic and apoptotic cells (early and late apoptosis) in a concentration dependent manner due the potential biological activities of *T. nudatum* [20].

5. CONCLUSION

In summary, this study reported that *T. nudatum* extract showed anticancer effects against human breast cancer cell lines (MDA-MB-231) *in vitro*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We conducted our research after obtaining proper approval.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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