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Research Article

In vitro antimicrobial, anticancer, and apoptosis-inducing effects of the methanolic extract of *Launaea mucronata*

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Abstract

Traditional medicine is widely used in the treatment and management of various ailments due to its low toxicity, low number of side effects and low cost. Many components of common fruits and vegetables play crucial roles as chemopreventive or chemotherapeutic agents. This study aimed to evaluate *in vitro* the antioxidant, cytotoxic and antimicrobial activities of *Launaea mucronata's* methanolic stems and leaves extract. In this screening study, *Launaea mucronata's* methanolic extracts showed remarkably antifungal activity against *Candida albicans*. The maximum zone of inhibition of the methanolic extract of *Launaea mucronata* leaves was detected against *Proteus vulgaris* with inhibition zones of 17.8 mm and 14.6 mm, respectively. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay results showed high antioxidant activity for the extract almost comparable to that of ascorbic acid at 30 µg/ml, which indicates that it might potentially be developed into a successful antioxidant agent. Meanwhile, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was performed by screening the extract against HepG2 (Hepatocellular), A549 (Lung), HCT116 (Colon) and MCF7 (Breast) cancer cells and it was found that the extract exerted its highest activity against A549 cells with an IC_{50} value of 14.9 µg/ml. The extracts also shown lower cytotoxic activity against normal, healthy MRC-5 cells, with IC_{50} values of 204.83 g/ml for the stem extract and 412.4 g/ml for the leaves extract, respectively. This suggests that the extract is safe for normal, healthy cells, which is an important characteristic of any possible anticancer treatment. The antiproliferative and apoptosis activities of our selected plant showed that the extracts induced S-phase arrest and apoptosis in A549 cells. This high cytotoxic activity of the extract indicates that highly bioactive pure compounds could potentially be isolated from the extract in future studies and further developed into an anticancer agent specifically against lung cancer. Therefore, the current study has proven the potential of *Launaea mucronata's* methanolic extract as a source of potent antioxidant and anticancer agent.

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From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Keywords: antioxidant; antimicrobial; anticancer agent; cytotoxic activity; DPPH and MTT assays; *Launaea mucronata*

Introduction

Natural products serve as a source of lead compounds that can be used in the process of drug discovery (Atanasov *et al.*, 2021). The genus Lacunae belongs to the largest and most common family of flowering plants known as Asteraceae, plant members that belong to this genus are widely distributed around the world, especially in the South Mediterranean, Africa, and Asia (Samia *et al.*, 2000; Michel *et al.*, 2020). It grows mainly in saline, sandy and dry habitats. The genus consists of around 54 species, of which 10 are in Saudi Arabia (Razag *et al.*, 2007). A number of species belonging to this genus have been documented to have biological activities as hypolipidemic, antioxidant, vasorelaxant, antithrombotic, non-steroidal anti-inflammatory and antitumor (Babelly *et al.*, 2016; Elsharkawy, 2017; Belmouhoub *et al.*, 2018; Sitarek *et al.*, 2020). *Launaea mucronata* is a species that belong to the genus *Launaea* and is characterized by having a dichotomously branched blue stem and long leaves (grey-green) (Mansour *et al.*, 1983; Zidorn *et al.*, 2007).

Launaea mucronata the is enriched with phytochemical constituents it was reported that the main phytochemical constituents of *Launaea mucronata* are flavonoids (quercetin, rutin, isorhamnetin and coumarins) oxygenated and non-oxygenated hydrocarbons, and terpenoids (Ahmed *et al.*, 2006; El-Sharkawy and Mahmoud, 2015).

The aim of this study is to assess *in vitro* antibacterial, antioxidant, as well as anti-neoplastic activity of the methanolic extract of *Launaea mucronata*.

Materials and Methods

Chemicals

All chemicals used were of high quality and analytical grade purchased from Sigma-Aldrich (MO, USA).

Collection and extraction of plant material

Launaea mucronata was obtained from Hail, Saudi Arabia. The plant material was identified by Dr. Majid, Prince Sattam bin Abdulaziz University, and a voucher specimen (No. COP001) was deposited at the College of Pharmacy, University of Hail. The air-dried powdered leaf and stem parts (1.2 kg each) were extracted separately by repeated cold maceration with 70% methanol (3 × 7 liters) to allow the extraction of phytoconstituents with different solubilities. The extracts were concentrated using vacuum to give 100 g and 70 g of residue of leaf part methanolic extract (LML) and root methanolic extract (LMS), respectively.

DPPH assay

The DPPH assay was used to assess the antioxidant properties of the extract. The methanolic extract was checked at different concentrations from 5-30 µg/ml and activities compared with ascorbic acid as a positive control (Viturro *et al.*, 1999). the reduction in colour was measured at 517 nm using double beam spectrophotometer (Unicam Helios Alpha, UK). The percentage of DPPH radical scavenging (I) was calculated as follows:

Inhibition (%) = 100 (Absorbance blank – Absorbance extract)/Absorbance blank

Hydrogen peroxide radical (H_2O_2) scavenging activity

The antioxidant activity of extract was determined by Hydrogen Peroxide Radical (H_2O_2) Scavenging Activity assays in triplicate and average values were considered. A solution of H_2O_2 (43 mM^{-1}) was prepared in

phosphate buffer (pH 7.4). A volume 0.2 mL of extract in distilled water (at different concentrations) was added to H_2O_2 solution (600 µL of 43 mM^{-1}). The absorbance of H_2O_2 at 230 nm was read after 10min for comparing with a blank solution containing phosphate buffer without H_2O_2 *(Ruch et al.*, 1989) using double beam spectrophotometer (Unicam Helios Alpha, UK). Butylated hydroxy toluene (BHT) was used as a reference standard. The H_2O_2 radical scavenging percentage of the extracts was calculated using the following equation:

% of inhibition = [(Ablank – Asample)/ Ablank] × 100 g extract.

Butylated hydroxy toluene used as a standard antioxidant and showed IC50 of 11.74 ± 0.54 µg/ml.

Cell culture

MCF7 cells (ATCC No. HTB-22™ human breast cancer cell line), HepG2 cells (ATCC No. HB-8064™ human Hepatocellular carcinoma cell line), A549 cells (ATCC CCL-185™ human lung carcinoma) and HCT116 cells (ATCC No. CCL-247™ human colon carcinoma cell line) were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37 ^oC in a humidified atmosphere with 5% CO_2 and were sub-cultured two to three times a week.

Cell viability assay

Tumour cell lines were suspended in medium at concentration 5×10^4 cell/well in Corning® 96-well tissue culture plates and then incubated for 24 hr. The tested compounds were then added into 96-well plates (three replicates) to achieve ten concentrations for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (Sun-Rise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as $[(OD_t/OD_c)] \times 100\%$ where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of untreated cells. The relation between surviving cells and drug concentration was plotted to get the survival curve of each tumour cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells.

Apoptosis analysis (Annexin V-FITC Assay)

Apoptotic cells were further analysed by Annexin V-FITC assay. Briefly, A549 (lung carcinoma) cells were cultured to a confluent monolayer then treated with the tested sample at the IC50 concentration as described earlier. After treatment for 72 h, the A549 cells were then harvested and rinsed twice in PBS (20 min. each) followed by binding buffer. Moreover, cells were re-suspended in 100 µL of kit binding buffer with the addition of 1 µL of FITC-Annexin V (Becton Dickinson BD Pharmingen TM, Heidelberg, Germany) followed by 40 min. incubation at 4 °C. Cells were then washed and re-suspended in 150 µL of binding buffer with the addition of 1 µL of 4′,6-diamidino-2-phenylindole, is a fluorescent stain (DAPI) (1 µg/mL in phosphate buffer solution (PBS)) (Invitrogen, Life Technologies, Darmstadt, Germany). Then, the cells were analysed using the flow cytometer fluorescence activated cell sorter (FACS) Calibur (BD Biosciences, San Jose, CA). (Eldehna *et al.*, 2018). The cell cycle analysis was performed using the Cycle TESTTM PLUS DNA Reagent Kit (Becton Dickinson Immuno-cytometry Systems, San Jose, CA). The A549 cells (treated with the tested sample or non-treated) were stained with pro-podium iodide stain according to the kit instruction. The flow cytometer was adjusted at 488 nm excitation line (Argon-ion laser or solid-state laser) and emission

collected at emission collected at 530 nm (green, FITC) and 575–610 nm (orange, PI). Cell-cycle distribution was calculated using Cell-Quest software (Becton Dickinson Immuno-cytometry Systems, San Jose, CA) (Eldehna *et al.*, 2017).

Gel electrophoresis and immuno-blot analysis of proteins (Western Blot)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate proteins based on their size. When coupled with western blotting (immunoblotting), both are typically used to determine the presence and/or relative abundance of a target protein in a sample containing a complex mixture of proteins. In this technique, total protein in each sample is loaded and electrophoretically separated by applying an electric current, which allows the proteins to migrate through the gel matrix. In order for the proteins to migrate through the gel, they are first denatured and negatively charged by exposure to a detergent such as SDS. A molecular weight marker that produces bands of known size is used to help identifying proteins of interest. After the protein components have been sufficiently separated, they can be transferred to a polyvinylidene fluoride (PVDF) membrane by applying an electric current to the gel so that the proteins migrate out of the gel onto the membrane. For detection of a specific protein on the membrane, a primary antibody against that protein is added to form a protein-antibody complex followed by the addition of a secondary antibody that binds to the complex through its antibody side. The secondary antibody is typically linked to an enzyme that produces luminescence upon the reaction with its substrate. The amount of the luminescence, directly proportional to the amount of the protein that reacted with the antibody, is captured by Biorad Imager (Burnette, 1981; Elnagar *et al.*, 2017; Aborehab *et al.*, 2021).

Antimicrobial activity

Antimicrobial activity was determined by the diffusion agar technique in Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar University, Cairo, Egypt (Rex *et al.*, 2009). Strains were obtained from the bacteria stock present at RCMB. Petri plates containing 20 ml of nutrient (for bacteria) or malt extract (for fungi), agar medium was seeded with 1-3 days cultures of microbial inoculums (standardized inoculums 1-2 × 107 cfu/ml 0.5 McFarland standard). Wells (6 mm in diameter) were cut off into agar and 100 µl of plant extracts were tested in a concentration of 5 mg/ml and incubated at 37 °C for 24 h (bacterial strains) and 25 °C for 7 days (fungal strains). The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the well. The positive control used for fungi was ketoconazole with minimum inhibitory concentration (MIC) 100 mg/ml, while positive control used for bacteria strains was gentamycin with MIC 4 mg/ml (Elsharkawy *et al.*, 2018).

Statistical analysis

Statistical analysis of difference between means was carried out using two-way analysis of variance 2-way ANOVA). In case of significant F-ratio, post-hoc Bonferroni's test for multiple comparisons was applied to evaluate the statistical significance between treatment groups at $P < 0.05$ level of significance. All the statistical analyses were done using Graph Pad prism statistical software version 7.

Results and Discussion

From literatures it was reported that *Launaea mucronata* is a species from *Launaea* Cass genus which are characterized with different secondary metabolites including terpenoids, steroids, triterpenoid saponin, sesquiterpene lactones, coumarins, flavonoids, flavone glycosides and phenolic compounds (Abdelkrim *et al.*, 2012). On this base, the antioxidant activity of methanolic extract of *Launaea mucronata* stem and leaves were determined using DPPH and hydrogen peroxide assay. The results showed a dose response profile of scavenging activities over a dose range from 0-40 µg/ml for methanolic stem and leave extract compared with ascorbic

standard in DPPH assay, IC50 of methanolic extract of leaves, stem and ascorbic were 15.33 µg/ml, 66.71 µg/ml and 0.06 µg/ml. Furthermore, the hydrogen peroxide scavengers' assay was carried out on the concentration ranges from 0-200 11.74 µg/ml. the results show that IC_{50} of methanolic extract of leaves, stem and butylated hydroxy toluene (BHT) is >200 µg/ml, 70.91 µg/ml and 11.74 µg/ml, respectively. Collectively, we could conclude that scavenger activity depends on type of assay conducted and dose range (Figure 1A and B).

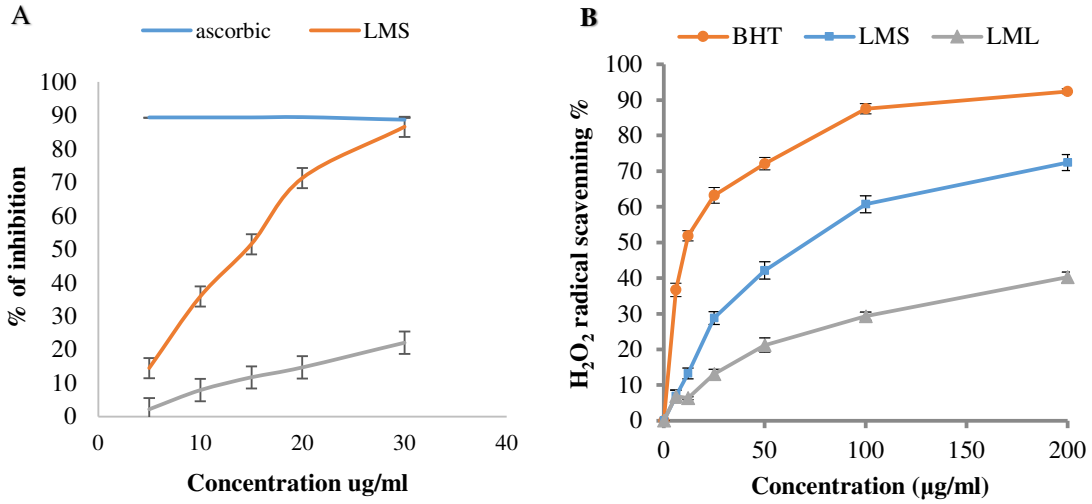

Figure 1. Dose response profile of antioxidant activities of methanolic extract using **(A)** DPPH assay with IC_{50}=0.06 µg/ml of ascorbic, 15.33 µg/ml for leave methanolic extract and 66.71 µg/ml for stem methanolic extract and **(B)**hydrogen peroxide assay with IC_{50}= 11.74 µg/ml for butylated hydroxy toluene, 70.91 µg/ml for methanolic stem extract and >200 µg/ml for leaves methanolic extract.

Launaea Cass genus has antitumor activities. The screening of the cytotoxic effect of *Launaea mucronata* extracts (stems and leaves) was performed using MTT cell viability assay. Four cancer cell lines were incubated with the extracts for 24 h and 72 h and the status of cell growth was observed (Figure 2A-2D). Moreover, the extracts' toxicity towards normal cell was assessed *via* screening them against normal healthy MRC-5 cells.

The statistical analysis carried out on the incubation period effect of both methanolic extracts on different tumour cell lines revealed that time exhibited a significance effect on result of IC50 obtained (LMS; F=1430.95: p<0.0001, LML, F=194.81: p<0.0001, Vin; F=1365.7: p<0.0001). These results in consistence with previous study. Methanolic extract of stem induced a significant inhibition of IC50 the cell viability following 24 hours of HepG2 (Hepatocellular), A549 (Lung), HCT-116 (Colon) and MCF-7 (Breast) cancer cells compared with respective treatment following 72 hours with IC_{50} values of HepG2 (IC_{50}, 276.7 and 40.5 µg/ml; p<0.0001), A549 (IC_{50}, 53.2 and 14.9 µg/ml; p<0.0001), HCT-116 (IC_{50}, 97.2 and 47.6 µg/ml; p<0.0001) and MCF-7 (IC_{50},109.4 and 55.7 µg/ml; p<0.0001) respectively. Methanolic extract of leaves induced a significant inhibition of IC_{50} the cell viability following 24 hrs. compared to 72 hrs. of HepG2 (IC_{50}: 148.1-56.9 µg/ml; p<0.0001, respectively), A549 (IC_{50}: 123.2-48.3 µg/ml; p<0.0001, respectively), HCT-116 (IC_{50}: 112.9-57.7 µg/ml; p<0.0001, respectively) and MCF-7 (IC_{50}: 180.8 -61.9 µg/ml; p<0.0001, respectively). The extracts of stem and leaves were also found to have lower cytotoxic activity against normal, healthy MRC-5 cells compared to vinblastine treatment following 24 hrs (IC_{50}, 204.83, 412.4 and 98.74 ± 3.51 µg/ml, p<0.0001, respectively) and 72 hrs (IC_{50}, 80.27, 112.00 and 43.00, p<0.0001, respectively). This indicates that the extracts low toxicity towards normal, healthy cells, which is a crucial property of any potential anticancer agent. From two-way analysis of variance, table 1 demonstrated that stems extract induced a significant inhibition of cell viabilities for all cell lines types compared to leaves extract following 24 and 72 hrs

incubation periods. It is crucial to note that, the cytotoxic activity of the extracts was comparable to that of Vinblastine Sulfate (positive control) with all cancer cell lines used, and that further confirms the significant bioactivity of the methanolic extract.

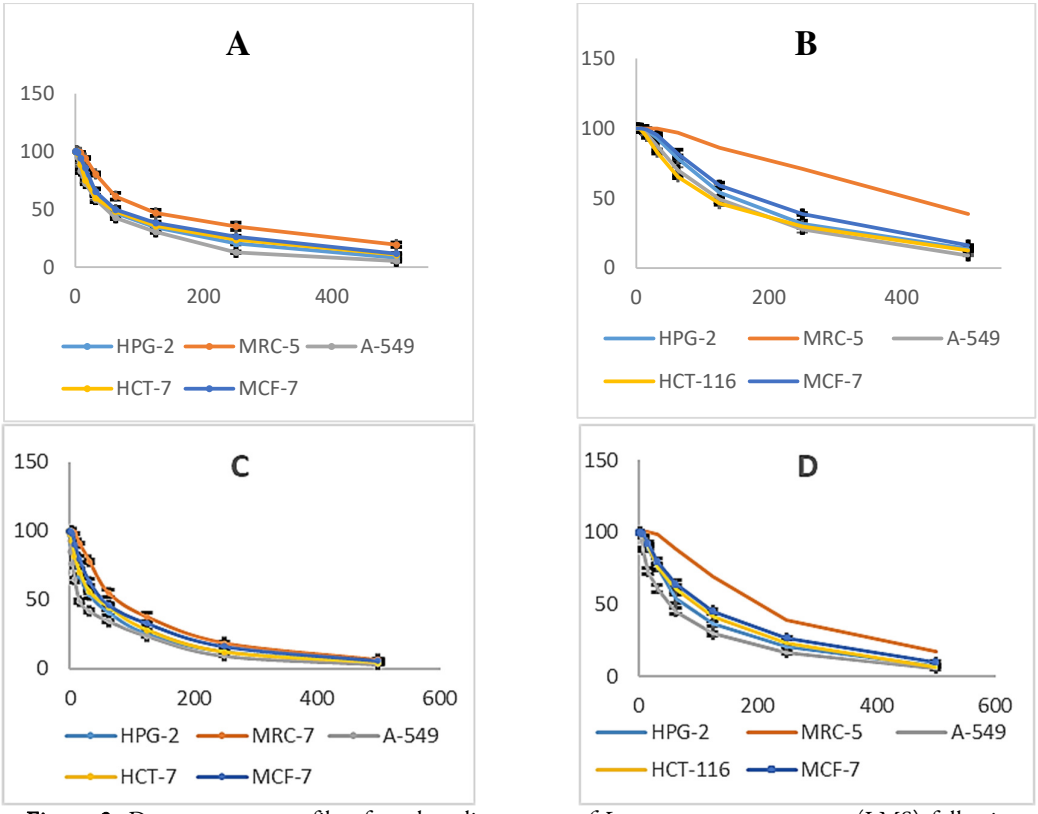

Figure 2. Dose response profile of methanolic extract *of Launaea mucronata* stem (LMS) following incubation period for 72 hr (A), 24 hr (B) and leaf (LML) following incubation period for 72 hr (C), 24 hr (D) on the cell growth viability of HepG-2, A-495, HCT-116, MCF-7 and MRC-5 cells lines. All data represented in mean ±SD, n=3

Table 1. Demonstrated IC_{50} values of *Launaea mucronata* methanolic extract against HepG2, A549, HCT116, MRC-5 and MCF7 cells

Treatment		IC_{50} (µg/ml)[1]				
Time	Group	HepG2	A549	HCT116	MCF7	MRC-5
24 hours	LMS	76.70 ± 6.4a	53.2 ± 5.1a	97.20 ± 7.8a	109.40 ± 9.2a	204.83 ± 7.31a
	LML	148.10± 10.7b	123.2± 9.6b	112.00± 9.5b	180.80± 13.4b	412.40 ± 11.48b
	Vin	2.90 ± 0.51c	24.6 ± 1.8c	3.50 ± 0.7c	5.90 ± 0.27c	98.74 ± 3.51c
72 hors	LMS	40.50 ± 2.9d	14.9 ± 1.3d	47.60 ±3.3d	55.7 ± 3.9d	80.27 ± 4.6d
	LML	56.90 ± 4.1e	48.30 ± 3.1e	57.70 ± 4.2e	61.90 ± 5.6e	112.00 ± 5.3e
	Vin	0.93± 0.21f	7.12 ± 0.38f	1.48± 0.38f	2.10 ± 0.46f	43.00 ± 2.09f

1 IC50 values are reported as mean IC50 ± SD, n=3, Different letters between treatments denote significant differences (Tukey test, $p < 0.001$).

Normal cell cycle is a set of events responsible for cell propagation or cell apoptosis through controller systems. Controller systems are checkpoints (G1/S and G2/M) maintain the fidelity of DNA replication, repair, and division. Checkpoints lead to cell arrest in both G1 and G2 phases if there is DNA damage or mis-

aligned chromosome at the mitotic spindle in this case (Pietenpol and Stewart, 2002; Zhang *et al.*, 2014). In attempt to explore the machinery of *Launaea mucronata* methanolic leave and stem extract as antitumor agent the levels of checkpoints were estimated to clarify the efficacy of the methanolic extracts to induce a cell rest and may enter the apoptotic phase. Lung cancer cell lines and IC_{50} concentration of both extracted were tested. The results showed that there was an increase in the percentage of cells in the S-phase relative to the control after treatment with the methanolic extract of stems (6.56% compared to 1.97%) and leaves (9.14% compared to 1.97%). Therefore, this indicates that the extracts induce S-phase cell-cycle arrest in A549 cells. It is also interesting to note that there was also an increase in the pre-G1 apoptotic phase after treatment with the stems (42.81% compared to 36.17%) and leaves (39.99% compared to 36.17%) extracts. This increase in the proportion of cells at the pre-G1 phase indicates that the extracts induced apoptosis in A549 cells which corroborates the data obtained from the Annexin V-FITC assay. Therefore, it can be deduced from the cell-cycle analysis data that the extracts induced S-phase arrest and apoptosis in A549 cells (Table 2, Figure 3).

Table 2. Effect of 48.3ug/ml of Leaves extract of *Launaea mucronata* (LML) and 14.9 ug/ml stem extract of *Launaea mucronata* (LMS) compared to control treatments on the phases of cell cycle of lung cancer cell line (A549)

Sample code	Tested conc. (µg/ml)	%G0-G1	%S	%G2-M	%Pre G1
Sample LML (Treated cells)	48.3	46.24	39.99	13.77	9.14
Sample LMS (Treated cells)	14.9	44.18	42.81	13.01	6.56
A549 cells (control)	0	51.88	36.17	11.95	1.97

Normal cells are characterized with a distinct morphological structure and biochemical features differ from necrotic or apoptotic cells. From cell cycle study on both methanolic extract on cell line lung cancer (A549), it was important to study the morphological structure of A549 cells treated with different methanolic extracts was carried out using Annexin-V FTIC assay as an apoptotic indicator. In this assay the degree of fluorescent and propidium iodide (PI) intensities identify the type of cell (Castedo *et al.*, 2002; Pozarowski *et al.*, 2003; Wlodkowic *et al.*, 2009). The result in figure 5 depicted that leaves extract of *Launaea mucronata* (LML) exhibited an increase in the percentage of Annexin V-FITC positive apoptotic cells relative to the control cells, including both the early (1.31% compared to 0.55%) and late (2.88% compared to 0.18%) (Table 2). There was also an increase in necrotic cells (4.95% compared to 1.24%). The stems extract of *Launaea mucronata* (LMS) exhibited an observable increase in the percentage of Annexin V-FITC positive apoptotic cells including both the early (0.94% compared to 0.55%) and late (2.27% compared to 0.18%) apoptotic phases. There were also some cells that demonstrated necrotic cells (3.35% compared to 1.24%) (Figure 4). From these results we can concluded that LML is a potent inducer for initiation the programmed cell death of lung cancer cells.

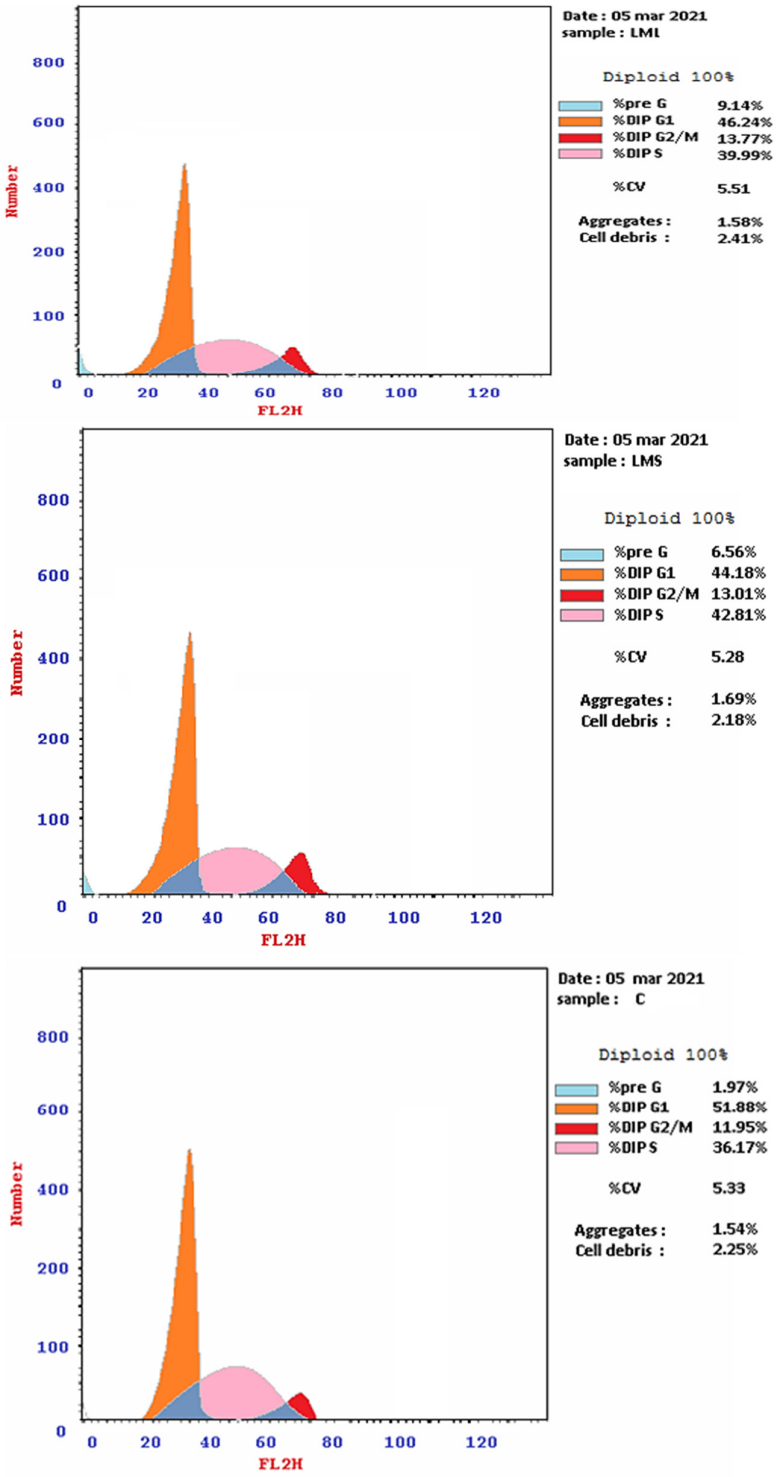

Figure 3. Photograph of flow cytometry analysis shows the effect of 48.3 ug/ml of leaves extract of *Launaea mucronata* (LML) and 14.9 ug/ml stem extract of *Launaea mucronata* (LMS) compared to control treatments on the phases of cell cycle of lung cancer cell line (A549)

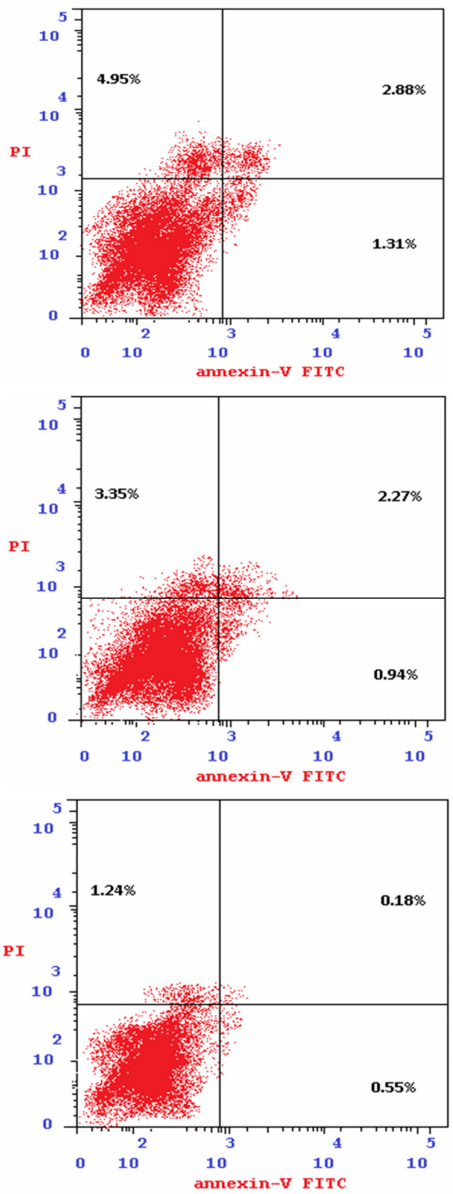

Figure 4. The effect of leaves methanolic extract (IC_{50}=48.3ug/ml) **(A)**, stem methanolic extract (IC_{50}= 14.9 ug/ml) **(B)** of *Launaea mucronata on* the percentage of Annexin V-FITC positive staining monolayer A549 cells versus control **(C)** using flow cytometry

To the level of morphological structure and checkpoints of cell cycle the antitumor efficacy of both methanolic extracts were tested. The level of biochemical characteristic of apoptotic cells which is regulated by several apoptotic factors, the expression of apoptosis-related proteins; pro-apoptosis protein, Bax, the anti-apoptosis protein, Bcl-2 and the tumour suppressor, p53 as well as caspase-3, as activation of caspases plays a significant role in programmed cell death were estimated (Ramadan *et al.*, 2019; Pisani *et al.*, 2020). results showed that Bax is up-regulated along with down-regulated of Bcl_2 upon treatment with leaves and stems extract of *Launaea mucronata*. The calculated value for the Bax/Bcl_2 ratio in comparison with that of the control compound revealed an upregulation by 2 and 5-fold for the stems and leaves extracts, respectively (Table 3). Moreover, leaves and stems extract of *Launaea mucronata* remarkably upregulated the expression level of cleaved caspase-3 by 2 and 3 folds in comparison with control cells, respectively. The p53 tumour

suppressor acts to integrate multiple stress signals into a series of diverse antiproliferative responses. One of the most important p53 functions is its ability to activate apoptosis, and disruption of this process can promote tumour progression (Pisani *et al.*, 2020). As indicated by the results in Table 3, the leaves extract of our selected plant increased the p53 level nearly 2-folds as compared with the control, however, there was no significant increase in p53 expression level after treatment with the stems extract (Figure 5). Collectively, from the biochemical results indicted that methanolic leaves extracts is a potent inducer of a protein apoptotic markers than methanolic extract these data confirmed the results obtained by Annexin-V FTIC assay. Previous studies demonstrated that *Launaea mucronata* enriched with phytonutrients

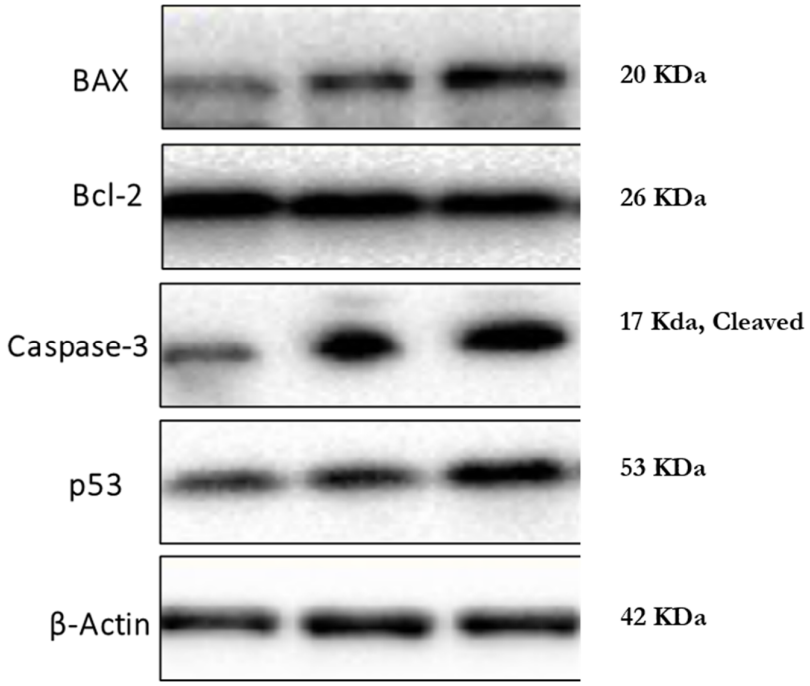

Figure 5. Showed the protein expression of Bcl_2, Bax, Caspase & P53 mediated apoptosis in lung cancer cell lines (A549) following treatments with methanolic extracts of leaves methanolic extract (IC_{50}=48.3 ug/ml), stem methanolic extract (IC_{50}= 14.9 ug/ml) of *Launaea mucronata*

Table 3. Effect of methanolic extracts of leaves and stem of *Launaea mucronata* on expression of apoptotic protein Protein expression of Bcl_2, Bax, Caspase & P53 Mediated Apoptosis in lung cancer Cell lines (A549)

Samples	Protein expression (normalized to β-actin)*				
	BAX	Bcl-2	BAX/Bcl-2 ratio	Caspases-3	p53
Control (A549 cells non-treated)	1.00	1.00	1.00	1.00	1.00
LMS (14.9 µg/ml)	1.35	0.71	1.90	2.54	1.24
LML (48.3 µg/ml)	2.01	0.42	4.78	3.86	1.74

* All the data are normalized to β-actin, and the values are given as fold changes from the control, which is set to '1'.

Antimicrobial activity

The antimicrobial activity of the leaves and stem extract of our selected plant was evaluated by measuring the zones of inhibition produced on a range of pathogenic microorganisms as shown in Table 6 using the diffusion agar technique. Limited inhibition zones were obtained, and generally the Gram-negative bacteria were more susceptible to the extract than the Gram-positive bacteria. The stem and leaves extracts demonstrate

high activity against *Proteus vulgaris* with inhibition zones of 17.8 mm and 14.6 mm, respectively. Moreover, the leaves extract was found to possess antifungal activity against *Candida albicans* with an inhibition zone diameter of 12.7 mm. For the other tested strains, leaves and stem extracts of *Launaea mucronata* showed weak to no activity. The antimicrobial activity of the extracts was further assessed quantitatively *via* measuring the extracts' minimum inhibitory concentration (MIC) using the microdilution method in microtiter plate. It was found that the leaves extract demonstrated antifungal activity against *Candida albicans* with an MIC value of 625 µg/ml, and that is actually comparable to that of the positive control ketoconazole, which possesses an MIC value of 312.5 µg/ml, reflecting the extract's potential as a potent antifungal agent. Moreover, the stem and leaves extract also showed activity against *Proteus vulgaris* with MIC values of 312.5 µg/ml and 625 µg/ml, respectively. There was no significant antimicrobial activity observed against the other strains investigated, similar findings have been reported for methanolic extract of *Launaea mucronata* (Gouda *et al.*, 2014). These results are in line with the data obtained from our diffusion agar assay (Tables 4, 5).

Table 4. Mean zone of inhibition in mm produced on a range of pathogenic microorganisms

Tested microorganisms	Sample Name		
FUNGI	**LMS**	**LML**	***Ketoconazole***
Aspergillus fumigatus (RCMB 002008)	NA	NA	19.2±1.2
Candida albicans RCMB 005003 (1) ATCC 10231	8.7±0.9	12.7±1.1	20.8±1.4
Gram Positive Bacteria	**LMS**	**LML**	***Gentamycin***
Staphylococcus aureus (RCMB010010)	NA	NA	25.1±1.63
Bacillus subtilis RCMB 015 (1) NRRL B-543	9.2±0.48	9.8±0.6	27.3±1.5
Gram Negative Bacteria	**LMS**	**LML**	***Gentamycin***
Escherichia coli (RCMB 010052) ATCC 25955	10.3±0.7	NA	29.7±1.9
Proteus vulgaris RCMB 004 (1) ATCC 13315	17.8±1.42	14.6±0.8	26.4±1.2

The sample was tested at 10 mg/ml concentration
The test was done using the diffusion agar technique, Well diameter: 6.0 mm
Positive control for fungi *Ketoconazole* 100 µg/ml
Positive control for bacteria *Gentamycin* 4µg/ml
*NA: No activity.

Table 5. The Antimicrobial activity as minimum inhibitory concentration (MIC) in µg/ml of tested microorganisms

Tested microorganisms	Sample Name		
FUNGI	**LMS**	**LML**	***Ketoconazole***
Aspergillus fumigatus (RCMB 002008)	-	-	156.25
Candida albicans ATCC 10231	10000	625	312.5
Gram Positive Bacteria	**LMS**	**LML**	***Gentamycin***
Staphylococcus aureus (RCMB010010)	-	-	9.7
Bacillus subtilis NRRL B-543	10000	5000	4.8
Gram Negative Bacteria	**LMS**	**LML**	***Gentamycin***
Escherichia coli ATCC 25955	10000	-	4.8
Proteus vulgaris ATCC 13315	312.5	625	4.8

The test was done using Microdilution method in Microtitre plate

Conclusions

In this study, the antimicrobial, cytotoxic and antioxidant activities of the methanolic extracts of *Launaea mucronata* 's stems and leaves were evaluated. The antimicrobial activity of plant extracts was evaluated with antibiotic susceptible and resistant microorganisms. Maximum zone of inhibition of methanolic leaves extract of *Launaea mucronata* was found only against *Proteus vulgaris*. It was found that the leaf extract showed antifungal activity against Candida albicans with comparable MIC value in compared to the positive control ketoconazole, which indicates that this extract could possess a promising antifungal activity. Antioxidant activity results showed that the extract displayed high antioxidant activity almost comparable to that of ascorbic acid, while good cytotoxic activity was also generally demonstrated by the extract against the tested cell lines with the highest activity being observed against A549 lung cancer cells. Moreover, there is a regulation of BAX genes, inducing apoptosis, downregulation of BCl2 gene in A549 cell line. Furthermore, this study-demonstrated inhibition of A549 lung cancer cells proliferation due to BAX gene upregulation and decreased BCl2 gene expression. Apoptotic study on this plant revealed that, the extracts caused S-phase arrest and apoptosis in A549 cells. The extract also showed negligible cytotoxic activity against normal MRC5 cells, if this indicates anything, it indicates that the extract is safe for normal cells, and this makes it a strong candidate treatment as an anti-cancer. Therefore, it can be concluded from these data that the extract has potential to be used as a natural antioxidant and a therapeutic agent against lung cancer. Further experiments are required to confirm the cytotoxic activity of the extract against lung cancer cells and explore its cytotoxic mechanism of action; furthermore, it is also crucial to attempt isolating bioactive pure compounds from this crude extract as it may eventually lead to a novel therapeutic agent against lung cancer.

Authors' Contributions

ASA had contributed to the study conception, design and Project administration. Plant was collected by DAA and GA. Materials were prepared by RUS. Data collection and analysis were performed by KMY. The data was interpreted by MKBB. The first draft of the manuscript was written by ENA and WAH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdelkrim C, Belboukhari M, Belboukhari N, Djeradi H (2012). Phytochemical and biological studies on *Launaea* Cass. genus (Asteraceae) from Algerian Sahara. Current Topics in Phytochemistry 11:67-80.

Aborehab NM, Elnagar MR, Waly NE (2021). Gallic acid potentiates the apoptotic effect of paclitaxel and carboplatin via overexpression of Bax and P53 on the MCF-7 human breast cancer cell line. Journal of Biochemical and Molecular Toxicology 35(2):e22638. *https://doi.org/10.1002/JBT.22638*

Ahmed Z, Ali D, Malik A (2006). Structure determination of ursene-type triterpenes by NMR techniques. Magnetic Resonance in Chemistry 44(7):717-719. *https://doi.org/10.1002/MRC.1803*

Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, Supuran CT (2021). Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery 20:200-216. *https://doi.org/10.1038/s41573-020-00114-z*

Babelly M,Abajy MY,Nayal R (2016). Hypolipidemic effect of *Achillea biebersteinii* ethanolic extract in hamsters with diet-induced hypercholesterolemia. International Journal of Pharmacognosy and Phytochemical Research 8:1555-1559.

Belmouhoub M, Chebout I, Iguer-ouada M (2018). Antidiabetic and anti-hypercholesterolemic effects of flavonoid-rich fractions of Rosmarinus officinalis in streptozotocin-induced diabetes in mice. Phytotherapie 16:204-210. *https://doi.org/10.3166/PHYTO-2018-0054*

Burnette WN (1981). "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate--polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Analytical Biochemistry 112:195-203. *https://doi.org/10.1016/0003-2697(81)90281-5*

Castedo M, Ferri K, Roumier T, Métivier D, Zamzami N, Kroemer G (2002). Quantitation of mitochondrial alterations associated with apoptosis. Journal of Immunological Methods 265:39-47. *https://doi.org/10.1016/S0022-1759(02)00069-8*

El-Sharkawy ER, Mahmoud K (2015). Cytotoxity of two new coumarin derivatives isolated from *Launaea mucronata*. Natural Product Research 30(4):394-398. *http://dx.doi.org/101080/1478641920151017492*

Eldehna WM, Abo-Ashour MF, Ibrahim HS, Al-Ansary GH, Ghabbour HA, Elaasser MM, ... Safwat NA (2018). Novel [(3-indolylmethylene)hydrazono]indolin-2-ones as apoptotic anti-proliferative agents: design, synthesis and in vitro biological evaluation. Journal of Enzyme Inhibition and Medicinal Chemistry 33:686-700 *https://doi.org/10.1080/14756366.2017.1421181*

Eldehna WM, Abo-Ashour MF, Nocentini A, Gratteri P, Eissa IH, Fares M, ... Supuran CT (2017). Novel 4/3-((4-oxo-5-(2-oxoindolin-3-ylidene)thiazolidin-2-ylidene)amino) benzenesulfonamides: Synthesis, carbonic anhydrase inhibitory activity, anticancer activity and molecular modelling studies. European Journal of Medicinal Chemistry 139:250-262. *https://doi.org/10.1016/J.EJMECH.2017.07.073*

Elnagar MR, Walls AB, Helal GK, Hamada FM, Thomsen MS, Jensen AA (2017). Probing the putative α7 nAChR/NMDAR complex in human and murine cortex and hippocampus: Different degrees of complex formation in healthy and Alzheimer brain tissue. PLoS One 12(12):e0189513. *https://doi.org/10.1371/journal.pone.0189513*

Elsharkawy ER (2017). Isolation of phytoconstituents and evaluation of anticancer and antioxidant potential of *Launaea mucronata* (Forssk.) Muschl. subsp. Pakistan Journal of Pharmaceutical Science 30(2):399-405.

Elsharkawy RE, Ed-dra A, Abdallah EM, Ali AMH (2018). Antioxidant, antimicrobial and antifeedant activity of phenolic compounds accumulated in *Hyoscyamus muticus* L. African Journal of Biotechnology 17:311-321. *https://doi.org/10.5897/AJB2017.16316*

Gouda Y, Abdallah Q, Elbadawy M, Basha AA, Alorabi AK, Altowerqe AS, Khaled, Mohamed M (2014). Cytotoxic and antimicrobial activities of some compositae plants growing in Taif area, Saudi Arabia. International Journal of Pharmaceutical Science Invention 3:43-48.

Mansour RMA, Ahmed AA, Saleh NAM (1983). Flavone glycosides of some *Launaea* species. Phytochemistry 22:2630-2631. *https://doi.org/10.1016/0031-9422(83)80189-7*

Michel J, Abd Rani NZ, Husain K (2020). A review on the potential use of medicinal plants from Asteraceae and Lamiaceae plant family in cardiovascular diseases. Frontiers in Pharmacology 11:1-26. *https://doi.org/10.3389/fphar.2020.00852*

Pietenpol JA, Stewart ZA (2002). Cell cycle checkpoint signaling: cell cycle arrest versus apoptosis. Toxicology 182:475-481. *https://doi.org/10.1016/S0300-483X(02)00460-2*

Pisani C, Ramella M, Boldorini R, Loi G, Billia M, Boccafoschi F, Volpe A, Krengli M (2020). Apoptotic and predictive factors by Bax, Caspases 3/9, Bcl-2, p53 and Ki-67 in prostate cancer after 12 Gy single-dose. Scientific Reports 10:1-10. *https://doi.org/10.1038/s41598-020-64062-9*

Pozarowski P, Huang X, Halicka DH, Lee B, Johnson G, Darzynkiewicz Z (2003). Interactions of fluorochrome-labeled caspase inhibitors with apoptotic cells: a caution in data interpretation. Cytometry Part A. The Journal of the International Society for Analytical Cytology 55:50-60. *https://doi.org/10.1002/CYTO.A.10074*

Ramadan MA, Shawkey AE, Rabeh MA, Abdellatif AO (2019). Expression of P53, BAX, and BCL-2 in human malignant melanoma and squamous cell carcinoma cells after tea tree oil treatment *in vitro*. Cytotechnology 71:461-488. *https://doi.org/10.1007/S10616-018-0287-4*

Razag A, Auzi A, Hawisa NT, Sherif FM, Sarker SD (2007). Neuropharmacological properties of *Launaea resedifolia*. Revista Brasileira de Farmacognosia/ Brazilian Journal of Pharmacognosy 17:160-165. *https://doi.org/10.1590/S0102-695X2007000200004*

Rex JH, Ghannoum MA, Alexander BD, Andes D, Brown SD, Diekema DJ, ... Walash TJ (2009). Method for antifungal disk diffusion susceptibility testing of yeasts. Approved Guideline—Second Edition. CLSI document M44-A2;29(17).

Ruch RJ, Cheng S Jun, Klaunig JE (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10:1003-1008. *https://doi.org/10.1093/CARCIN/10.6.1003*

Samia R,Ashraf M,Shahida B,Rubeen A (2000). Antibacterial and antifungal activities of *Launaea nudicaulis* (Roxb.) and *Launaea resedifolia* (Linn.). Pakistan Journal of Biological Sciences 3:630-632. *https://dx.doi.org/10.3923/pjbs.2000.630.632*

Sitarek P, Merecz-Sadowska A, Śliwiński T, Zajdel R, Kowalczyk T (2020). An *in vitro* evaluation of the molecular mechanisms of action of medical plants from the Lamiaceae family as effective sources of active compounds against human cancer cell lines. Cancers 12:1-47. *https://doi.org/10.3390/CANCERS12102957*

Viturro C, Molina A, Schmeda-Hirschmann G (1999). Free radical scavengers from *Mutisia friesiana* (Asteraceae) and Sanicula graveolens (Apiaceae). Phytotherapy Research 13:422-424. *https://doi.org/10.1002/(SICI)10991573(199908/09)13*

Wlodkowic D, Skommer J, Darzynkiewicz Z (2009). Flow cytometry-based apoptosis detection. Methods in Molecular Biology 559:19-32. *https://doi.org/10.1007/978-1-60327-017-5_2*

Zhang L, Zheng Y, Deng H, Liang L, Peng J (2014). Aloperine induces G2/M phase cell cycle arrest and apoptosis in HCT116 human colon cancer cells. International Journal of Molecular Medicine 33:1613-1620. *https://doi.org/10.3892/IJMM.2014.1718/HTML*

Zidorn C, Ellmerer E-P, Heller W, Greil R, Guggenberger M, Ongania KH, ... Stuppner H (2007). A new sesquiterpene lactone sulfate from *Reichardia gaditana* (Asteraceae). Zeitschrift für Naturforschung 62:132-134. *https://doi.org/10.1515/znb-2007-0121*