



INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Characterization Of Therapeutic Leads From Plants Extracts Their Effect On Breast Cancer Cell Lines

1Pavithra Dasari, 2SWATHI, 3Prof; Nirmala Baburao, 4Prof:Anupalli Rojarani****

1RESEARCH SCHOLAR , 2RESEARCH SCHOLAR, 3PROFESSOR , 4PROFESSOR

1OSMANIA UNIVERSITY, HYDERABAD.TS,

2OSMANIA UNIVERSITY, HYDERABAD.TS,

3OSMANIA UNIVERSITY, HYDERABAD.TS,

4OSMANIA UNIVERSITY, HYDERABAD.TS

Abstract:

Plant-derived herbal merchandise are giant sources for drug discovery and improvement which includes considerable potentials in stopping and coping with oxidative stress, making them promising applicants in most cancers and different sickness therapeutics. Their results had been related to phytochemicals along with phenolic compounds and their antioxidant activities. The abundance and complexity of those bio-ingredients spotlight the want for well-described in vitro characterization and quantification of the plant extracts/arrangements which could translate to in vivo results and with a bit of luck to scientific use. This evaluate article seeks to offer applicable facts approximately the applicability of cell-primarily based totally assays in assessing anti-cytotoxicity of phytochemicals thinking about numerous conventional and cutting-edge methods.

Keywords: medicinal plants; Cell culture extracts; antioxidant activity; cell lines; cytotoxicity; apoptosis; bio printing.

INTRODUCTION

Cancer is one of the main reasons of loss of life worldwide. It is the primary or 2d main reason of loss of life previous to age 70 in 112 of 183 international locations and 1/3 or fourth main reason in a in addition 23 international locations, in line with World Health Organization (WHO) estimates in 2019 [1]. The growing prominence of most cancers as a main reason of loss of life in mixture with restrained medical interventions surely compromises the results of remedy on populace traits in most cancers mortality, even in evolved international locations [2]. Although a mixture of screening and remedy is step by step powerful in decreasing mortality from a few cancers, an predicted worldwide most cancers burden of 28.4 million instances in 2040, a upward thrust of 47% from 2020 values, necessitates the improvement of recent equipment to deal with the unmet desires in most cancers management [1]. Although newer, greater particular remedies are displaying promising results, they may be expensive, and in addition studies is needed to decide the way to first-rate use those drugs, in addition to the toxicities related to their use [3].

Cells cultured in the lab can be classified into three different types: **primary cells, transformed cells, and self-renewing cells**. Plant Cell cultures are able to synthesizing a huge array of phytochemicals which can be used as pharmaceuticals, meals components and beauty elements. Although maximum researches have targeted at the manufacturing of pharmaceutically essential compounds, during the last decade there was a tremendous improve withinside the improvement of plant mobileular way of life technology used for manufacturing of lively beauty elements or meals components [1-3]. Large-scale cultivation of undifferentiated and differentiated plant mobileular cultures is connected to numerous problems of technological, financial and legislative nature. However, in vitro increase of plant cells, tissue and organ cultures beneathneath a managed surroundings is the maximum promising green era for sustainable deliver of precious phyto-elements even with the aid of using rare, endemic, protected, threatened or endangered plant species. Today, regardless of full-size efforts, most cancers nevertheless stays an competitive killer global. Moreover, over the last decade, novel artificial chemotherapeutic dealers presently in use clinically have now no longer succeeded in pleasing expectancies regardless of the full-size price in their improvement. Therefore there's a consistent call for to expand new, powerful, and lower priced anticancer drugs [3].

From the sunrise of historic medicine, chemical substances derived from flora were used to deal with human diseases. Natural merchandise have acquired growing interest during the last 30 years for his or her ability as novel most cancers preventive and healing dealers [4, 5]. In parallel, there's growing proof for the ability of plant-derived compounds as inhibitors of diverse degrees of tumorigenesis and related inflammatory processes, underlining the significance of those merchandise in most cancers prevention and therapy. Approximately 60% of medication presently used for most cancers remedy were remoted from herbal merchandise [6] and the plant state has been the maximum huge source. These consist of vinca alkaloids, Taxus diterpenes, Camptotheca alkaloids, and Podophyllum lignans. Currently, of sixteen new plant-derived compounds being examined in scientific trials, thirteen are in section I or II and 3 are in

section III [7]. Among those compounds, flavopiridol, remoted from the Indian tree *Dysoxylum binectariferum*, and *meisoindigo*, remoted from the Chinese plant *Indigofera tinctoria*, were proven to show off anticancer consequences with lesser toxicity than traditional drugs [7]. Medicinal flora represent a not unusual place opportunity for most cancers remedy in many nations across the world [8, 9]. At this time, extra than 3000 flora global were mentioned to have anticancer properties. Globally, the occurrence of plant-derived merchandise for most cancers remedy is from 10% to 40% with this charge attaining 50% in Asiatic patients [9–11]. The 3 simplest plant extracts have been then decided on for added studies focusing additionally on the character of cell loss of life due to those plant extracts. Our speculation turned into that complete mobileular extracts would possibly include a couple of molecules with antitumor sports and be very powerful in killing human most cancers cells. Plant cells way of life era has the ability to satisfy the constantly developing call for for bioactive herbal compounds withinside the close to future. Currently, while selecting their ingredients and cosmetics, maximum human beings choose to use herbal merchandise with low ecological footprints. In line with this tendency, the previous few years have visible an exponential growth withinside the quantity of cosmetics with lively materials acquired with the aid of using plant cell way of life era [3, 4]. The extensive look for improvements and improvement of recent merchandise with a couple of precise sports are the riding forces at the back of the fast development within side the improvement of plant cell way of life-derived lively elements for the wishes of the cosmetics industry. Plant extracts investigated have been *Urtica membranacea* (Urticaceae) (called extract quantity five withinside the study), *Artemisia monosperma* (Asteraceae) (called extract quantity 10), and *Origanum dayi* Post (Labiatae) (called extract quantity 11). All flora have been investigated as a part of the Middle Eastern Medicinal Plant Project (MEMP), an initiative of The Natural Medicine Research Center (NMRC) committed to the ethnobotanical investigation, domestication, conservation, and reintroduction and targeted screening of Israeli medicinal flora [13]. All 3 decided on plant extracts exhibited dose- and time-established killing talents in diverse human derived hematological and strong tumor mobileular traces and in number one cultures mounted from patients' biopsies. The killing hobby turned into precise closer to tumor cells, because the extracts had no impact on number one cultures of wholesome human cells (lymphocytes and fibroblasts). Several experiments have been completed to signify the plant extracts' sports. Using diverse strategies it turned into discovered that, the use of complete plant extracts, mobileular loss of life turned into precipitated through apoptosis. Plant extract five additionally verified robust anticancer talents with the aid of using inhibiting real tumor development in a breast adenocarcinoma mouse model. Our consequences advise that complete plant extracts are promising anticancer reagents.

Materials and Methods

2.1. Plant Selection. Plants were selected for screening using a focused method based on ethnobotanical information derived from The Natural Medicine Research Center's (NMRC) database of medicinal plants containing the traditional and folk medicine uses of over 500 Israeli species and from data mining techniques using additional sources including medieval pharmacopeias and medical encyclopedias translated from original Latin, Hebrew, and Arabic texts. Selected vegetation have been accumulated from wild reassets or harvested from domesticated vegetation derived from seeds acquired from wild vegetation. Domestication became done on the MEMP cultivation webweb page in Kibbutz Ketura, in Israel's southern Arava region (arid and wilderness species) and at Moshav Noam at the coastal plain (Mediterranean species). Plants have been cultivated with out the usage of fertilizers or insecticides the usage of shaded internet homes and drip irrigation. Collection of untamed vegetation became done together with Ms. Hagar Leschner and Dr. Ori Fragman-Sapir. All plant cloth became definitively diagnosed through both Dr. Ori Fragman-Sapir or Ms. Hagar Leschner and voucher herbarium specimens deposited on the Jerusalem Botanical Gardens.

2.2. Plant Extracts (Ethanol Extraction). Freshly harvested vegetation have been air-dried at room temperature and extracted with ethanol (50% v/v, 10 v in keeping with gram weight) through full of life stirring in a blanketed beaker for twenty-four h at room temperature and then the procedure became repeated. The supernatant of each extracts became filtered and ethanol evaporated in a chemical hood for four days with the evaporated extract frozen at -70° C accompanied through lyophilization until dryness. The dried extract became saved at four $^{\circ}$ C. Stock answers of the plant extracts (200 mg/mL) have been organized through weighing the powder and dissolving it in 10% DMSO/PBS. The answer became divided to aliquots and saved at -20° C, till used.

2.3. Cell Lines. The human most cancers cell lines HepG2, OVCAR three, 293, A549, T24P, and SU-DHL-1 and mouse cell lines D122 and B16 have been grown in DMEM supplemented with 10% fetal calf serum (FCS), 1 mM L-glutamine, one hundred units/mL penicillin, and one hundred μ g/mL streptomycin. The human most cancers cell lines Hec1A, Karpas, HUT102, Colo205, LNCaP, MCF-7, YC, and OSTR have been grown in RPMI 1640 supplemented with 10% S and antibiotics as above. E0771 mouse cells have been grown in F-12 (HAM'S) medium containing 10% S, 1 mM L-glutamine, 10 mM HEPES, and antibiotics as above. Primary human most cancers cultures have been grown in RPMI 1640 medium containing 1% nonessential amino acids, 1% sodium pyruvate, 1% sodium bicarbonate, 20% S, 10 μ M β -mercaptoethanol, 1 mM L-glutamine, and antibiotics as above. All media and dietary supplements have been bought from Biological Industries (Bet Ha'emek, Israel). The cells have been saved in a humidified environment with five% CO₂ at 37 $^{\circ}$ C. All cultures have been examined for mycoplasma infection and have been determined to be negative.

2.4. Isolation of Human Primary Lymphocytes from Healthy Donors. Human peripheral blood mononuclear cells (PBMCs) from healthful donors (acquired from the Blood Bank-Hadassah Medical Center) have been removed on a FicollIsopaque gradient (density 1.077; Sigma-Aldrich, St. Louis, MO, USA). The removed PBMCs have been then counted and checked for viability the usage of Trypan blue and used on an man or woman basis. To spark off the lymphocytes, all mononuclear cells have been grown in RPMI 1640 supplemented with 10% (v/v) FCS, 2 mM L-glutamine, 100 units/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, 10 μM β -mercaptoethanol, and phytohemagglutinin (PHA 20 $\mu\text{g}/\text{mL}$) for three days. The activated cells have been washed and resuspended withinside the presence of 10 units/mL of recombinant IL-2 (PeproTech EC Ltd., London, UK) to hold cell viability. Naïve lymphocytes have been grown in RPMI 1640 supplemented with 10% (v/v) FCS, 2 mM Lglutamine, 100 units/mL penicillin, one 100 $\mu\text{g}/\text{mL}$ streptomycin, and 10 μM β -mercaptoethanol.

2.5. Primary Human Cancer Cultures. Fresh tissue specimens have been taken from most cancers sufferers present process healing debulking procedures. All tissue specimens have been washed numerous instances with Leibovitz (L15) medium, minced, and subjected to enzymatic proteolysis for 2 h at 37° C with mild shaking in Leibovitz medium containing collagenase kind I (200 units/mL), hyaluronidase (100 units/mL) (Sigma-Aldrich), penicillin (1000 units/mL), streptomycin (1 mg/mL), and amphotericin B (2.5 Tissue arrangements have been centrifuged for 10 min at 200 g, and the pellets have been suspended in RPMI 1640 medium containing all dietary supplements and plated in 100 mm petri dishes. After 1–3 weeks, whilst the cultures had reached a density of 8×10^6 cells/plate, histopathological diagnoses and cell viability assays (see below) have been carried out.

2.6. Cell Viability. 104 /100 μL /nicely cells developing in suspension or 5×10^3 /100 μL /nicely adherent cells have been seeded and handled with growing concentrations of the plant extracts for 48–72 h, and then Cell Titer-Blue reagent (Promega, Madison, WI, USA) became introduced in line with the manufacturer’s commands to decide cell All remedies have been carried out in triplicate.

2.7. Cell Cycle Analysis through Propidium Iodide (PI) Staining. 0.3×10^6 /3.5 mL Hec1A cells have been handled with the plant extracts (1.5 mg/mL, very last concentration), for 4 to 48 h. Samples have been eliminated for assaying cell viability, and the final cells have been centrifuged at 500 g for 6 min at 4° C, washed with bloodless PBS, and resuspended in 0. 5 mL of PI hypotonic answer (50 $\mu\text{g}/\text{mL}$ PI; 0.1% sodium citrate; 0.1% triton X100). After in a single day incubation at 4° C, cell cycle evaluation of the cells became carried out the usage of FACScan and the Cell Quest program (Becton Dickinson).

2.8. In Vitro Caspase three Activity Assay. 105 /100 μL /properly Colo205 cells have been handled with the plant extracts (1.5 mg/mL, very last awareness), for 24–48 h. Caspase three hobby inside the cells

became assessed with the aid of using Apo-ONE Homogeneous Caspase 3/7 Assay Kit (Promega). Experiments have been achieved in parallel with cell viability assays.

2.9. Western Blot Analysis. Samples have been separated on 12% SDS-PAGE gels. The proteins have been then electro transferred onto Immobilon-P switch membrane (Millipore, Millipore, Bradford, MA, USA) and blotted with anti-PARP (Cell Signaling Technology, Inc, Boston, USA; 1 : 1,000). Band visualization became carried out the use of a more advantageous chemiluminescence kit (ECL, Biological Industries).

2.10. DNA Laddering. 0.5×10^6 /3 mL Colo205 cells have been seeded and the plant extracts have been brought (1.5 mg/mL, very last awareness) for 24, 48, or 72h. The cells have been then collected, washed two times with PBS, resuspended in four mL of lysis buffer (15 mM Tris-HCl pH 7.4, 3 mM EDTA pH 8.0, one 150 mM NaCl, 0.2% SDS, 10 μ g/mL proteinase K, and 50 μ g/mL RNase), and incubated over night time at 37° C. DNA became then extracted with the aid of using the subsequent procedure: four mL phenol/chloroform (1 : 1 ratio to lysis buffer extent) became brought, and the answer became centrifuged at 3150 g for 5 min at room temperature. The top segment became remoted and 1 : 1 extent of chloroform became brought. The answer became then centrifuged once more below the equal situations and the top segment became collected. NaCl awareness became adjusted to 0.5M. Two volumes of absolute bloodless (-20° C) ethanol have been brought and the answer became incubated at -70° C for 1 h, to permit the DNA precipitate to form. The precipitate became then remoted with the aid of using centrifugation at 10,000 g for 30 min at 4°C. The pellet became washed two times with 70% bloodless ethanol, air-dried, and resuspended in 10 mM Tris, 1 mM EDTA, pH 8.0. The very last awareness became measured with the aid of using nanodrop machine. 10 μ g DNA of every pattern became loaded on a 1.5% agarose gel.

2.11. Real-Time PCR Analysis of Apoptosis-Related mRNAs in Colo205 Treated Cells. 0.5×10^6 /3.5 mL Colo205 cells have been handled with 1.5 mg/mL (very last awareness) of extracts 5, 10, and eleven for 3–24 h. Total mRNA became extracted and reversetranscribed into cDNA the use of Verso cDNA kit (Thermo Specific, Epsom, Surrey, UK). Individual mRNA ranges have been quantified the use of Real-Time PCR (Applied Biosystems, Foster City, CA, USA). Each 2 μ L pattern contained 1 μ L primers (10 ng), 10 μ L SYBR Green (Applied Biosystems), and seven μ L H₂O in a complete extent of 20 μ L in step with pattern. The primers have been as follows:

Bax (exons 4-5, 116 bp) Sense: TCT GAC GGC AAC TTC AA CTG;

Antisense: CAG CCC ATG ATG GTT CTGA;

Bcl2 (exons 2-3, 134 bp)

Sense: CCC CTG GTG GAC AAC ATC;

Antisense: CAG CCA GGA GAA ATC AAA CAG;

Caspase 3 (exons 7-8, 133 bp)

Sense: GAA CTG GAC TGT GGC ATT GA;

Antisense: CCT TTG AAT TTC GCC AAG AA;

G6PD (exons 6-7, 283 bp)

Sense: TCT ACC GCA TCG ACC ACT ACC;

Antisense: GCG ATG TTG TCC CGG TTC.

The data was analyzed by the primer express program (Applied Biosystems)

2.12. Effect of Plant Extract 5 on Breast Cancer Growth in Mice. C57BL/6JolaHsd mice were radiated with 500RAD/5 GREY, left for one day to recover, and then subcutaneously injected with 105 E0771 cells, in the lower back. Treatment started one day after tumor induction and consisted of daily i.p. injections of 150 μg (in 300 μL) extracts 5, for 12 days. Control mice were injected with an equivalent volume of DMSO/PBS at the same concentration as in extract at the end of the experiment, mice were sacrificed; tumors were removed and weighed.

Results

3.1. Screening Plants for Their Anticancer Activity. 17 plants were initially selected and tested for anticancer activity based on their historical and traditional use in treating cancer. Whole plant extracts (ethanol extracted) were prepared, coded as plant extract numbers 1–17, and tested for their potential as anticancer agents. We examined quite a few most cancers cell traces originating from numerous human tumors which include hematopoietic tumors including T cell lymphoma and leukemia, in addition to stable tumors including colon, renal, breast, bladder, endometrial, and others (Table 1). This becomes accomplished via way of means of carefully tracking the viability of cultured human cells uncovered to the plant extracts. The plant extracts (introduced at 3mg/mL, very last concentration) inhibited the increase of the numerous human tumor cell traces ultimately main to cell loss of life. Efficacy of cell loss of life varied, relying at the particular plant extract (Figure S1 A–C in Supplementary Material to be had on-line at Specificity of plant extracts closer to most cancers cells become additionally evaluated. Normal human lymphoblast (EBV transformed) cells (OSTRA and YC cells; Table 1) have been now no longer suffering

from the remedy with the plant extracts (Figure S1 A–C). Thus, the plant extracts are cytotoxic to set up human most cancers cell traces however don't have any impact on wholesome human cells. Based on repeated assays, the 3 only anticancer plant extracts, 5, 10, and 11, have been selected for in addition studies.

Table 1: Human tumor cells used in the study.

Cell Line	Type
LNCaP	Prostate adenocarcinoma
Colo205	Colon carcinoma
Hec-1A	Endometrial adenocarcinoma
OVCAR 3	Ovarian carcinoma
HepG2	Hepatocellular carcinoma
MCF-7	Breast ductal carcinoma
293	Embryonic kidney adenocarcinoma
Karpas 299	T cell, non-Hodgkin's lymphoma
A549	Alveolar basal epithelial adenocarcinoma-lung
SU-DHL-1	Anaplastic large cell lymphoma
YC	Normal EBV transformed lymphoblasts
OSTRA	Normal EBV transformed lymphoblasts
HUT 102	T cell lymphoma
T24P	Urinary bladder carcinoma

3.2. Plant Extract Induced Cell Death Was Time- and Concentration Dependent. The three simplest anticancer plant. extracts 5, 10, and eleven, had been first examined for his or her impact on most cancers cell strains at decrease concentrations. As may be visible in Figure 1, the 3 plant extracts inhibited cell boom additionally at decrease concentrations, with one-of-a-kind efficacy relying at the particular plant extract and the most cancers cell line examined. Inhibition of cell boom become additionally time-established, as established in Figures 2(a) and 2(b). Plant extracts 5, 10, and 11 eleven had been examined additionally on number one lymphocytes from wholesome manipulate donors and confirmed no killing interest on those cells (Figure 2(c)). Thus, plant extracts 5, 10, and 11 exhibited time- and concentration-established anticancer interest at the same time as having no impact on number one cell cultures from wholesome humans.

3.3. Effect of the Plant Extracts on Primary Cultures Obtained from Cancer Patients. To look at the cytotoxicity of our plant extracts towards cells comparable to the authentic in vivo tumors as intently as possible, number one cultures had been installed from biopsies of one-of-a-kind most cancers patients (colon carcinoma and breast most cancers patients). As may be visible in Figures 2(d) and 2(e), extract 5 affected cell boom very quickly, as become visible with the installed cell strains. Extracts 10 and 11 brought on cell loss of life greater slowly; however, their killing interest accelerated seventy two h after the begin of treatment. It have to be cited that despite the fact that mobileular boom of the number one mobileular cultures become inhibited with the aid of using approximately 60%, this killing interest is lots decrease than that measured in installed mobileular strains (see Figure S1 and 1-2). This can be defined with the aid of using the reality that number one cultures are maximum likely now no longer homogenous however alternatively a blended populace of cells, along with wholesome regular cells for which the plant extracts had been now no longer cytotoxic.

3.4. Mechanism of Cytotoxicity

4.1. Effect of Plant Extracts on Cell Cycle of Hec1A Endometrium Cancer Cells. In past due levels of apoptosis, cells break up to shape apoptotic bodies. Each apoptotic frame incorporates simplest a part of the authentic cell's DNA content. When stained with PI, this populace is called the sub-G1 populace and is mosomes. In addition, apoptotic cells reveal particular morphological modifications which include chromatin condensation and plasma membrane blebbing. These modifications motive the mobileular to be greater granular and large in length whilst analyzed with the aid of using FACS. In order to illustrate the impact of our plant extracts at the cell extracts (1.5mg/mL each, very last concentration) for one-of-a-kind time intervals and analyzed with the aid of using FACS. Results are proven in Figure three. Extract five brought on ~13% boom withinside the sub-G1 cell populace after 24 h. This boom become observed with the aid of using a 14%crease in cells at G1 phase (Figure three(a)). Extract eleven brought on adramatic boom (40%) in cell numbers at G2 without a obvious alternate within side the sub-G1 mobileular populace (Figure three(b)). Extract 10 brought on an boom withinside the sub-G1 mobileular populace (9.4% boom, Figure three(c)), a decrease (from 43.26% to 20.79%) in cells at G1 and a large boom (from 25.26% to 39%) in cells at G2 (Figure three(c)). These effects imply that extracts five and 10 motive traditional apoptotic cell loss of life, at the same time as extract eleven is inflicting loss of life thru a one-of-a-kind mechanism.

4.2. Effect of Plant Extract five on Breast Cancer Growth in Mice. In order to check the impact of the extracts in an in vivo mouse version, we first checked their impact on 3 mouse most cancers cells: B16 melanoma, D122 lung carcinoma, and E0771 breast carcinoma cells. Plant extract five become determined to be the simplest on E0771 cells (effects now no longer proven) and as a consequence become selected to be examined in vivo within side the E0771 breast most cancers version in mice. First the nonspecific

toxicity of extract five become examined on male C57BL/6JolaHsd mice with the aid of using unmarried i.p. injections of growing quantities of extract five. The mice had been accompanied for three weeks for essential symptoms and symptoms and weighed two times a week. Injecting as much as a 150 μ g of plant extract five become determined to be secure without a poisonous consequences at the mice. Next, C57BL/6JolaHsd mice had been injected with E0771 mouse breast most cancers cells and dealt with with extract five (see methods, Section 2.12). As established in Figure 6(a), tumors of mice dealt with with extract five weighed approximately 50% much less than the ones of mice receiving simplest DMSO/PBS (vehicle). Figure 6(b) suggests the tumors from both groups, emphasizing the substantial distinction in tumor length of the dealt with mice in comparison to the manipulate mice. Thus, plant extract five inhibits tumor boom in vivo, in a mouse breast most cancers version.

Conclusions

Whole cell extracts (ethanol extraction) from *Urtica membranacea* (Urticaceae), *Artemisia monosperma* (Asteraceae), and *Origanum dayi post* (Labiatae), flowers indigenous to the coastal undeniable and wasteland regions of Israel, exhibited doseand time-established killing talents on numerous human derived hematological and stable tumor cell strains and number one cultures installed from patients' biopsies. The killing interest become particular closer to tumor cells, because the plant extracts had no impact on number one cultures of wholesome human cells. Cell loss of life due to the complete plant extracts become thru apoptosis. Plant extract from *Urtica membranacea* confirmed especially robust anticancer talents because it inhibited real tumor development in a breast adenocarcinoma mouse version. Our effects advise that complete plant extracts are promising anticancer reagents.

References

- [1] D. J. Newman, "Natural products as leads to potential drugs: an old process or the new hope for drug discovery?" *Journal of Medicinal Chemistry*, vol. 51, no. 9, pp. 2589–2599, 2008.
- [2] D. J. Newman, G. M. Cragg, and K. M. Snader, "Natural products as sources of new drugs over the period 1981–2002," *Journal of Natural Products*, vol. 66, no. 7, pp. 1022–1037, 2003.
- [3] M. Gordaliza, "Natural products as leads to anticancer drugs," *Clinical and Translational Oncology*, vol. 9, no. 12, pp. 767–776, 2007.
- [4] M. Khan, A. A. Mousa, K. V. Syamasundar, and H. Z. Alkhatlan, "Determination of chemical constituents of leaf and stem essential oils of *Artemisia monosperma* from central Saudi Arabia," *Natural Product Communications*, vol. 7, no. 8, pp. 1079–1082, 2012.
- [5] M. Stavri, K. T. Mathew, T. Gibson, R. T. Williamson, and S. Gibbons, "New constituents of *Artemisia monosperma*," *Journal of Natural Products*, vol. 67, no. 5, pp. 892–894, 2004.
- [6] C. Formisano, D. Rigano, A. Russo et al., "Phytochemical profile and apoptotic activity of *Onopordum cynarocephalum*," *Planta Medica*, vol. 78, no. 15, pp. 1651–1660, 2012.
- [7] M. Stavri, C. H. J. Ford, F. Bucar et al., "Bioactive constituents of *Artemisia monosperma*," *Phytochemistry*, vol. 66, no. 2, pp. 233–239, 2005.