Patel *et al. Beni-Suef Univ J Basic Appl Sci* (2022) 11:43
https://doi.org/10.1186/s43088-022-00203-5

Beni-Suef University Journal of Basic and Applied Sciences

REVIEW Open Access

Phyto-factories of anti-cancer compounds: a tissue culture perspective

Poonam Patel[1*], Vaibhav Patel[2], Arpan Modi[3], Sushil Kumar[1] and Yogesh M. Shukla[4]

Abstract

Background: Cancer is one of the most critical but ubiquitous causes of death grappled from past decades. Widely used chemotherapy with cytotoxic activity blocks/ kills the cancer cell. The compounds targeted for anticancerous activity are either derived synthetically or naturally (through plants or microbial origin). Current day, versatile role of plants in medicinal field has been attributed to the secondary metabolites it produces, known for their anticancer activity. Therefore, discovery, identification and commercial production of such novel anticancer drugs is escalated and are centerpiece for pharmaceuticals.

Main body: A biotechnological approach, principally tissue culture, leads the candidacy to be an alternative method for production of anticancer compounds. A wide range of bioactive agents like alkaloids, steroids, phenolics, saponins, flavonoids, and terpenoids are in huge demand commercially. Plant tissue culture applications are constructively more advantageous over conventional methods in terms of their continuous, controlled, aseptic production, large scale and de novo synthesis opportunity. Various bioreactors are used for mass cultivation of bioactive compound at commercial level. For example: stirred tank reactors are used for production of shikonin from *Lithospermum erythrorhizon,* vincristine from *Catharanthus roseus*, podophyllotoxin from *Podophyllum* etc. Strategies like callus culture, suspension culture and hairy root culture are opted for mass cultivation of these bioactives.

Conclusions: This review summarizes plant tissue culture as a promising strategy proven to be a colossal breakthrough in reliable and continuous production of existing and novel anticancer compounds and help in combating the increasing future demands.

Keywords: Anticancer compounds, Plant tissue culture, Bioreactors, Mass cultivation

1 Background

Cancer is defined as uncontrolled growth of a group of cells, which invade surrounding tissues/organs and lead to destruction of normal cells. It is one of the most fatal diseases worldwide and is the second major cause of death after heart diseases. According to Global cancer statistics in 2018, 18.1 million new cases of cancer were reported with 9.6 million deaths. Lung cancer was most common and leading cause of death (18.4%) followed by breast cancer (11.6%), prostate cancer (7.1%) and colorectal cancer (6.1%) [23]. Treatments using chemopreventive measures and radiation therapy are common in use but are painful to tolerate. These treatments should be highly effective but in the present scenario, adverse side effects and resistance to radiation or drug treatments have overpowered its benefits. Hence, there is an urge for targeting an alternative strategy which can slow down, prevent or cure carcinoma. Plants and their derived compounds are thoughtful substituent in relation to cancer with respect to their high bio-accessibility, less side effects, safer in use and importantly are cost effective [3]. There are more than 1500 plant derived anticancer compounds which can be developed as potential drugs are under research and about five hundred drugs are under clinical trials. Thus, it is an imperative prerequisite to

*Correspondence: poonam.kanani2009@gmail.com
[1] Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat, India
Full list of author information is available at the end of the article

develop potent and less noxious drugs for fighting cancer with its ever increasing demand. As the plant-based compounds are obtained from natural sources, which in turn lead to extinction of those sources.

Nowadays, tools in biotechnology-based techniques offer production of bio-active compounds from plants in laboratories without disturbing the natural ecosystem. It has opened a pavement for production of these active compounds directly via, using plant cells, tissues or organs by aseptically growing and through genetic improvement to obtain desirable product [34]. This review summarizes, present scenario of naturally derived anticancer compounds and plant cell culture techniques as an alternative strategy for the production of same.

Typically, about 80% of global human population is driven by using traditional medicines for its primary health preventive measures back from three decades. Plants, therefore, are proven sources of best medicinal practices; extensive research is en-route to find natural aspects for control and cure of cancer. The continuous and enormous use of herbal remedies has widely been escalated with domination in countries like the UK, Europe, North America, Australia, Africa, India as well as other developing countries are also seeking for these alternatives over the chemical drugs [32]. The current status on market of plant-based medicines forecast the uplift by 5.88% (annual growth rate) in period of 2018–2023 with inclination of consumers demand toward source of plant-based medicinal products. The report provides outlooks into the major leading companies which play eminent role in herbal medicine sector are: Bayer AG, ZeinPharma Germany GmbH, Arkopharma, Dasherb Corp, Hishimo Pharmaceuticals, Blackmores, BEOVITA, Dr. Willmar Schwabe India Pvt. Ltd., Schaper&Brummer, Himalaya Global Holdings Ltd, Venus Pharma GmbH and Arizona Natural Products [62].

Till now the studies in field of cancer, had focused to rule out the deleterious effects of cancer via targeting the vital mechanism playing role behind regulation of cancer cells which affect locally or systemically. And finally through identification, isolation, characterization, studying their effects, a particular anticancer agent is selected and undergoes clinical trials.

1.1 Anticancer compounds

In general anticancer compounds by definition are the compounds or agents with potent activity to act against or arrest the growth of cancer. In an overview from 1980s and till now, around 174 potent anticancer compounds have been approved commercially, with 53% (93 drugs) accounted for natural based or derived products [12]. Normally, the anticancer compounds are categorized into two types: chemical compounds and natural compounds.

1.1.1 Chemical compounds

Compounds such as alkylating agents (cisplatin), antimetabolites (5-Florouracil, 6-mercaptpurine), antibiotics (bleomycin, dactinomycin) etc which are used in chemo-treatments usually affecting the dividing cancer cells as well as normal cells during the course of treatment. Mostly all chemical cytotoxic agents have severe side effects, majority of them targets bone marrow, gastrointestinal tract, gonads (sex organs) and skin (hair follicle cells) etc.[24]. Also, these conventional drugs limits its usability by their unsuitable oral route application, less solubility in water, inadequate specificity, severe side effects and short half-life of compounds in body [51].

1.1.2 Natural compounds

Compounds which are derived from plants, microbes and animals are referred as natural compounds. These compounds provided naturally, have an interesting perspective towards development of novel outcomes for cancer treatment. Interestingly drugs developed from natural compounds such as vinblastine and vincristine (*Catharanthus roseus*), camptothecin (*Camptotheca*), paclitaxel/Taxol (*Taxus* sp.) and podophyllotoxin (*Podophyllum* sp.) and others are proven anticancer targets. In actual fact, almost half of the globally accepted drugs are nature-based, -derived or -small molecules [51].

Commercially available anticancer drugs are broadly classified into various categories based on their mechanism of action: alkylating agents, antimetabolites, antitumor antibiotics and topoisomerase inhibitors, hormone and hormone antagonists, natural therapy and other medications (Fig. 1) [99].

1.2 A better outlook with natural medicines!

The traditional uses of natural medicines for healing purposes are mentioned since ancient times. Common applications of these medicines included curing fever, cough and cold, irritable bowel syndrome, menstrual problems, wounds, stings, burns, ulcers, anxiety and depression [112]. Scientists have extensively researched for extracting potential anti-cancer compounds from plants which could be applied in preparation of drugs [11]. World health organization (WHO) has reported that, there are certain populations that rely completely on medicines from tropical plants as major source for treatment of diseases [27]. Boy et al. [22] reported that, around 35,000–70,000 plant species are targeted as drugs. Hence, the usage of plants for isolation of anticancer drugs had played a pivotal role in drug discovery. It is also critical to ensure that plant derived cytotoxic compound has a combination of desired effects such as: maximum potency against cancer and minimum deleterious effects on normal cells.

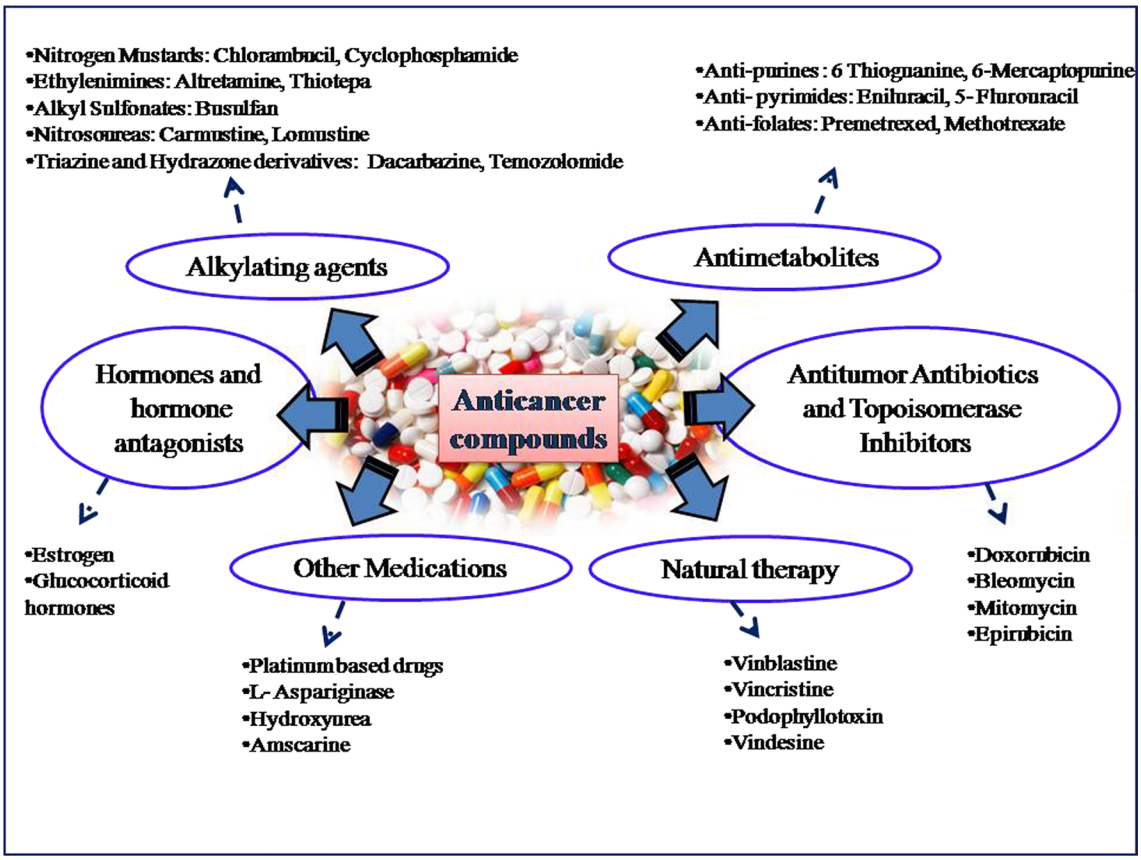

Fig. 1 Classification of anticancer compounds

1.3 Methodology for anti-cancer drug development

Conventionally anticancer drug discovery and development were primarily based on cytotoxic agents which were basically chemical compounds, as majority of other drugs. For example, the use of folate analogues for therapy of acute lymphoblastic leukemia was developed in 1948 [17]. On the other hand, the underlying mechanism of action playing role in inhibition of dihydrofolate reductase, was observed by Osborn et al. in 1958 [81]. Likewise, for nitrogen mustard, mustine, was already in use before its mode of action was even known. Recently the process of drug discovery and development has become very systemic and methodological, focusing on the insights of cancer at molecular level and specifically on target-based drugs. They are pre-processed for modification / inhibition of selected molecular targets. In recent years there have been various novel drugs approved by Food and Drug Administration (FDA) enlisted in Table 1 from 2010 to 2019. Anticancer compounds, as like other drugs, also undergo different stages in developmental paradigm before their marketing approval by the regulatory. The stages for potential drug development are as follows (Fig. 2):

1. Identification of potential anticancer compounds: It is accomplished through chemical synthesis or by natural extraction of targeted compound. This stage comprises identification of promising compounds, exploring its physical properties (such as solid state form, stability, melting point and solubility) and testing its potential under conditions of physiological stress at cellular level as expected in malignancy. Various other studies like ADME (absorption, distribution, metabolism and excretion) profile and those defining the best dose for the compound, toxicological profile, interaction profile with other drugs and its effectiveness are being conducted. Shortlisted compounds based on pre-mentioned criterion are scaled up in further developmental phases for formulation, which is guided by selected mode of administration in humans and its subsequent commercialization post approval.
2. Drug screening and preclinical trials: This stage involves confirmation of safety aspects of a drug

Table 1 FDA approved cancer drugs from 2010 to 2020

Drug name	Treatment	Approval status	Company name
Halaven (eribulin mesylate)	Breast cancer	Nov 2010	Eisai
Herceptin (trastuzumab)	Gastric cancer	Oct 2010	Genentech
Jevtana (cabazitaxel)	Prostate cancer	Jun 2010	Sanofi aventis
Abstral (fentanyl sublingual tablets)	Cancer pain in opioid-tolerant patients	Jan 2011	ProStrakan
Lazanda (fentanyl citrate) nasal spray	Cancer pain	Jun 2011	Archimedes
Vandetanib (vandetanib)	Thyroid cancer	Apr 2011	AstraZeneca
Xalkori (crizotinib)	ALK + non-small cell lung cancer	Aug 2011	Pfizer
Zytiga (abiraterone acetate)	Prostate cancer	May 2011	Centocor Ortho Biotech
Cometriq (cabozantinib)	Metastatic medullary thyroid cancer	Nov 2012	Exelixis
Abraxane (paclitaxel protein-bound particles for injectable suspension)	Non-small cell lung cancer,	Oct 2012	Celgene
Perjeta (pertuzumab)	First-line treatment of HER2 + metastatic breast cancer	June 2012	Genentech
Afinitor (everolimus)	HER2-negative breast cancer	July 2012	Novartis
Cometriq (cabozantinib)	Metastatic medullary thyroid cancer	Nov 2012	Exelixis
Stivarga (regorafenib)	For the treatment of previously treated patients with metastatic colorectal cancer	Sept 2012	Bayer HealthCare Pharmaceuticals
Subsys (fentanyl sublingual spray)	Treatment of breakthrough cancer pain	Jan 2012	Insys Therapeutics
Xtandi (enzalutamide)	Metastatic castration-resistant prostate cancer	Aug 2012	Medivation
Zaltrap (ziv-aflibercept)	Treatment of metastatic colorectal cancer	Aug 2012	Sanofi-aventis
Gilotrif (afatinib)	Metastatic non-small cell lung cancer with EGFR mutations, Approved July 2013	July 2013	Boehringer Ingelheim
Kadcyla (ado-trastuzumab emtansine)	Treatment of HER2-positive metastatic breast cancer	Feb 2013	Genentech
Xofigo (radium Ra 223 dichloride)	Treatment of prostate cancer with bone metastases	May 2013	Bayer Healthcare Pharmaceuticals
Cyramza (ramucirumab)	Treatment of gastric cancer	April 2014	Eli Lilly
Avastin (bevacizumab)	Treatment of persistent, recurrent, or metastatic cervical cancer	Aug 2014	Genentech
Avastin (bevacizumab)	Treatment of stage III or IV epithelial ovarian, fallopian tube, or primary peritoneal cancer	Nov 2014	Genentech
Lynparza (olaparib)	Treatment of previously treated BRCA mutated advanced ovarian cancer	Dec 2014	AstraZeneca
Zykadia (ceritinib)	Treatment of ALK + metastatic non-small cell lung cancer	April 2014	Novartis
Lonsurf (trifluridine and tipiracil)	Treatment of metastatic colorectal cancer	Sept 2015	Taiho Oncology
Onivyde (irinotecan liposome injection)	Treatment of metastatic pancreatic cancer following gemcitabine-based therapy	Oct 2015	Merrimack
Ibrance (palbociclib)	Treatment of HR-positive, HER2-negative breast cancer,	Feb 2015	Pfizer
Alecensa (alectinib)	Treatment of ALK-positive, metastatic non-small cell lung cancer	Dec 2015	Roche
Keytruda (pembrolizumab)	Treatment of PD-L1 positive advanced non-small cell lung cancer	Oct 2015	Merck
Lonsurf (trifluridine and tipiracil)	Treatment of metastatic colorectal cancer and metastatic gastric or gastroesophageal junction adenocarcinoma	Sept 2015	Taiho Oncology
Onivyde (irinotecan liposome injection)	Treatment of metastatic pancreatic cancer following gemcitabine-based therapy	Oct 2015	Merrimack
Opdivo (nivolumab)	Treatment of metastatic squamous non-small cell lung cancer	March 2015	Bristol–Myers Squibb
Portrazza (necitumumab)	Treatment of metastatic squamous non-small cell lung cancer	Nov 2015	Eli Lilly
Tagrisso (osimertinib)	Treatment of EGFR T790M mutation positive non-small cell lung cancer	Nov 2015	AstraZeneca

Table 1 (continued)

Drug name	Treatment	Approval status	Company name
Alecensa (alectinib)	Treatment of ALK-positive, metastatic non-small cell lung cancer	Dec 2015	Roche
Keytruda (pembrolizumab)	Treatment of PD-L1 positive advanced non-small cell lung cancer	Oct 2015	Merck
Opdivo (nivolumab)	Treatment of metastatic squamous non-small cell lung cancer	March 2015	Bristol–Myers Squibb
Portrazza (necitumumab)	Treatment of metastatic squamous non-small cell lung cancer	Nov 2015	Eli Lilly
Tagrisso (osimertinib)	Treatment of EGFR T790M mutation positive non-small cell lung cancer	Nov 2015	AstraZeneca
Syndros (dronabinol oral solution)	Tomiting associated with cancer chemotherapy	July 2016	Insys Therapeutics
Rubraca (rucaparib)	Treatment of advanced ovarian cancer in women with deleterious germline or somatic BRCA mutation	Dec 2016	Clovis Oncology
Keytruda (pembrolizumab)	Treatment of head and neck squamous cell cancer	Aug 2016	Merck
Tecentriq (atezolizumab)	Treatment of urothelial carcinoma and metastatic non-small cell lung cancer	May 2016	Genentech
Keytruda (pembrolizumab)	Treatment of microsatellite instability-high or mismatch repair deficient solid tumors and colorectal cancer	May 2017	Merck
Kisqali (ribociclib)	Treatment of breast cancer	March 2017	Novartis
Verzenio (abemaciclib)	Treatment of HR + , HER2-breast cancer	Sept 2017	Eli Lilly
Zejula (niraparib)	Treatment of recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer	March 2017	Tesaro
Alunbrig (brigatinib)	Treatment of advanced ALK-positive metastatic non-small cell lung cancer	April 2017	Ariad Pharmaceuticals
Imfinzi (durvalumab)	Treatment of advanced or metastatic urothelial carcinoma and Stage III non-small cell lung cancer	May 2017	AstraZeneca
Keytruda (pembrolizumab)	Treatment of urothelial carcinoma (bladder cancer)	May 2017	Merck
Nerlynx (neratinib)	Treatment of HER2 breast cancer	July 2017	Puma Biotech
Keytruda (pembrolizumab)	Treatment of recurrent or metastatic cervical cancer	June 2018	Merck
Talzenna (talazoparib)	Treatment of deleterious germline BRCA-mutated HER2-negative locally advanced or metastatic breast cancer	Oct 2018	Pfizer
Erleada (apalutamide)	Treatment of prostate cancer	Feb 2018	Janssen Oncology
Keytruda (pembrolizumab)	Treatment of recurrent or metastatic cervical cancer	June 2018	Merck
Lorbrena (lorlatinib)	Treatment of ALK-positive metastatic non-small cell lung cancer	Nov 2018	Pfizer
Opdivo (nivolumab)	Treatment of advanced small cell lung cancer	Aug 2018	Bristol–Myers Squibb
Opdivo (nivolumab)	Treatment of MSI-H or dMMR metastatic colorectal cancer	Aug 2018	Bristol–Myers Squibb
Talzenna (talazoparib)	Treatment of deleterious germline BRCA-mutated HER2-negative locally advanced or metastatic breast cancer	Oct 2018	Pfizer
Vizimpro (dacomitinib)	Treatment of metastatic non-small cell lung cancer	Sept 2018	Pfizer
Erleada (apalutamide)	Treatment of prostate cancer,	Feb 2018	Janssen Oncology
Herceptin Hylecta (trastuzumab and hyaluronidase-oysk)	Treatment of HER2-overexpressing breast cancer,	Feb 2019	Halozyme
Piqray (alpelisib)	Treatment of HR + , HER2-negative, PIK3CA-mutated advanced or metastatic breast cancer	May 2019	Novartis

Table 1 (continued)

Drug name	Treatment	Approval status	Company name
Tecentriq (atezolizumab)	Treatment of triple negative breast cancer	March 2019	Genentech/Roche
Keytruda (pembrolizumab)	Treatment of stage III non-small cell lung cancer,	April 2019	Merck
Tecentriq (atezolizumab)	Treatment of extensive-stage small cell lung cancer	March 2019	Genentech/Roche
Balversa (erdafitinib)	Treat adult patients with locally advanced or metastatic bladder cancer	April 2019	Janssen Products, LP
Piqray (alpelisib)	Treatment of breast cancer	May 2019	Novartis
Polivy (polatuzumab vedotin-piiq)	Treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma	June 2019	Genentech
Brigatinib	Anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC)	May 2020	ARIAD Pharmaceuticals Inc
Olaparib	First-line treatment for advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer	May 2020	AstraZeneca
Pembrolizumab	Hodgkin lymphoma (Chl)	Oct 2020	Merck Sharp & Dohme Corp
Nivolumab + ipilimumab	Treatment for unresectable malignant pleural mesothelioma	Oct 2020	Bristol-Myers Squibb Co

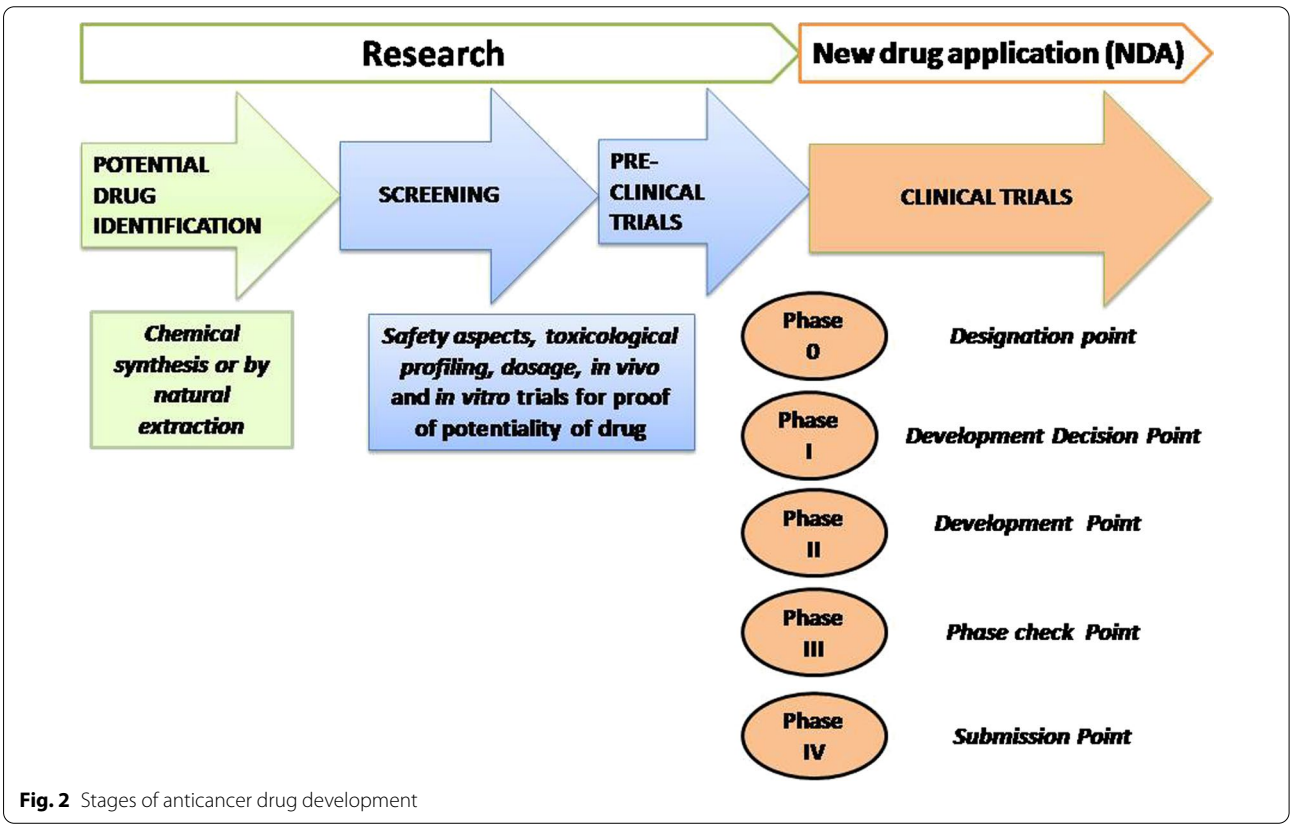

Fig. 2 Stages of anticancer drug development

for use in humans and detailed toxicological profile. Studies are being carried out in vivo (within the organism) and in vitro (a setting outside the organism) during the preclinical trials. This phase focuses mainly upon the safety aspects of the drug and its levels which can prove to be toxic to humans. After keen review of preclinical trial results, the decision is

made, whether the new compound can be tested in humans or not [53].

3. Clinical trials: They are a type of research study which explores the safety and efficacy of a treatment, strategy or a device by following strict regulations with a purpose of protecting the patients, parallelly producing reliable results which could be easily reproduced. Clinical trials comprise of various phases:

Phase 0 (Especially carried out for cytotoxic drugs)- These are typically "first in human studies". Study population is targeted to cancer patients, approx. 10-15 subjects per study. Sub therapeutic doses of the drug are administered for a maximum up-to 7 days. Focus of phase 0 is to select the 'lead candidate' by mainly looking at the bio-distribution (pharmacokinetics) and confirming whether the effects on the molecular target replicate the ones produced by the preclinical studies.

Phase I—For drugs other than cytotoxic agents, these are "first in human" studies. Phase-1 established recommended phase 2 dose of a drug, (RP2D) and collects data about the adverse effects in human subjects. It also includes details about pharmacokinetics (effect of body on the drug) and pharmaco-dynamics (effects of drug on the body) of the drug. Generally, the study population includes normal adult volunteers but occasionally the studies may target specific patient populations. It establishes MTD (maximum tolerated dose) and the dose range for a drug. Interestingly, phase-1 cancer trials include specific dose escalations in addition to the above-mentioned details. The escalations are basically of 3 types: 3+3 cohort (traditional), accelerated titration, and model guided (continuous reassessment). Typically, phase one for any drug consists of 20-100 subjects and as per an estimate 70% of drugs move on to phase 2.

Phase II—These are initial pilot studies involving several hundreds of subjects. Basic goal is to achieve preliminary efficacy data and details of side effects in human subjects (patient population). They can be further classified into phase 2a and phase 2b studies. 2a studies are pilot studies to understand drug efficacy in selected populations. Dose range of a drug is determined by testing multiple doses. While, 2b studies are well controlled efficacy trials with a definitive dose and provide a "Go/No go" decision. As per an estimate 33% of the phase 2 drugs move on to phase 3.

Phase III—These are actual pivotal trials carried out in larger population (300–3000 subjects) depending on diseased state. The main aim of phase 3 is to confirm the efficacy in large population and to monitor all the adverse effects encountered. Typically phase 3 studies last from one to several years. Again, it is estimated that 25% of drugs move on to next phase.

Phase IV—These studies are better known as 'post marketing surveillance'. Goal of this phase is to monitor the long-term side effects of a drug. Data are collected from consumers post approval and marketing [28].

2 Plant tissue culture: a new vision for cancer drug development

2.1 Introduction

Plant tissue culture is a technique of plant propagation under controlled environment (also known as micropropagation). The primary objective of the micropropagation is to establish aseptic conditions so that only plant cell will grow and other biotic factors such as bacteria, yeasts and fungi can be eliminated. Due to its intrinsic potential for growth (known as totipotency), any plant cell from any organ of the plant can be nurtured under in vitro conditions, which ultimately gives rise to whole new plant. This is achieved through the maintenance of physical factors such as light, humidity, pH of the medium and temperature and chemical factors such as macro and micro-nutrients, sugars, vitamins, plant growth regulators and gelling agents. The process of micropropagation starts with establishment of axenic culture (obtained from any plant parts) and ends with the hardening of the tissue culture raised plantlets. In between these two process there are steps of organogenesis. Organogenesis can be divided in to two type's viz., direct organogenesis in which the new shoot is emerged from the meristamatic cells such as shoot tips or nodes, or non-meristamatic cells such as leaf or internode; and indirect organogenesis in which the new shoot is emerged from the undifferentiated mass (callus) or through somatic embryogenesis. The whole process ends up with the development of true-to-type plants ready to transfer to the field. Now the basic question would arise that why the need of tissue culture in plant production when they can be cultivated (and subsequently the product can be harvested) on field? Here, the advantages of micropropagation are that the process is season independent (where on field it needs the right time to cultivate); it can be done in relatively less space and time; and the production of disease free superior quality plantlets [45]. However, the process for the plant production is costly so that it should be noted that not all the plants are suitable for the commercial production, rather, the selection of plant species to be micropropagated should be based on the issue they are

facing during vegetative or seed-based propagation. These issues include limited numbers of offshoots as in case of Banana [47]; dioecious nature of the plants in cases of Date palm and Papaya [63, 66]; and less viabilities of the seeds in case of Stevia [67].

Plants have always been most effective and lifesaving entity in the world of drugs for human population. Commonly, the bioactive compounds or secondary metabolites from plants are used as pigments, dyes, food additives, drugs, cosmetics, pesticides, perfume etc. [15]. Generally, plants modulate themselves by using their bizarre feature of plasticity when they fall victim to biotic and abiotic stresses. This lead plant to synthesize secondary bioactive molecules through activation of elicitors which evoke mechanism for production of these kind of secondary biomolecule. Mainly, for biotic stress: elicitors are categorized as exogenous elicitors such as chitin, glucans, chitosan which are pathogen induced, whereas the endogenous elicitors are pectin, pectic acid, polysaccharides induced by plants due pathogen attack. In contrast, there are abiotic elicitors such as: physical agents—heat, cold, atmospheric pressure, UV light; chemical agents: antibiotics, fungicides, pesticides, salts, heavy metals, ethylene. Thus elicitors regulates the gene expression, induces enzyme synthesis, promote formation of various secondary metabolites like alkaloids, flavonoids, phenylpropanoid, terpenoids, thionins and polypeptides [31].

In plants these compounds are not only vital for adaptation in relation to environmental fluctuations, but also have significant role as pharmaceutical products. Many plant species in nature are empowered with a range of curative properties, which are now being unraveled for plethora of applications in medical background. Numerous compounds in nature are already identified and proven to be effective against cancer. But the future demands of increasing population and a fewer availability of other alternatives has led to a potential and more eco-friendly approach (*i.e.* tissue culture) for production of anticancer drugs from plants.

Recent advances in biotechnology aided with plant tissue culture methodology have provided new insights into commercial production of anticancer drugs [31]. Various strategies in tissue culture can be utilized for enhancing production of natural cytotoxic compounds like: screening and selection of high production cell lines, optimization of media for growth and development of targeted bioactive compound etc. Plant tissue culture is the technique through which such important anticancer compounds can be produced under controlled conditions with less occupancy of space. Not only these bio-active compounds but also some of the therapeutic proteins have been produced commercially with the use of plant tissue culture. As compared to other cell-based production system for the production of therapeutic proteins, plant cell-based system has advantages with low to medium production costs; high scale-up capacity; highly purified products; very low contamination risk; and comparatively less cost for the product purification [39, 108]. Production of recombinant antibodies such as Human HIV antibody in Tobacco BY-2 cells [44]; recombinant proteins like Human epidermal growth hormone in Tobacco [75]; and recombinant vaccine such as Hepatitis B in Potato [85] have been reported using plant cell culture system.

2.2 Screening and selection of high production cell lines

Exploring preparatory material (initial material from the plant for procuring high production cell lines) which is directly correlated with highest anticancer activity (anticipated/studied) is the initial prerequisite in this strategy. The screening techniques are principally based on two conventional methods:

2.2.1 Cell-based bio-assay

The National Cancer Institute (NCI) screening was based on human tumor cell lines to search, compounds that obstruct cell growth. The program was initiated for plants, back in 1956. It covers novel active compounds, their botanical classification and its relationship with antitumor activities [71]. During initial years, the program was based on in vitro cytotoxicity assay which mainly involved three murine tumors (carcinoma 755, L1210 leukemia and sarcoma 180). But it had certain flaws like lack of identification of targeted compounds and high incidence of cells losing its viability. Hence, improved methods were developed which were primarily based on in vivo screening by P388 leukemia and later by 9ASK astrocytoma in in vitro condition [54]. Further advanced system was developed with human cell line-KB (human epidermoid carcinoma of the nasopharynx) cell culture [76], a primary indicator for determining the potent activity of compounds. As a matter of fact, human cell lines have higher sensitivity as compared murine leukemia cell lines (L1210 and P388, or in vivo assessment).

2.2.2 High-throughput screening

It involves compounds that hinder specific enzymes or proteins in vitro (phosphatases, kinases etc.). This approach is grounded on establishment of in vitro assay based on significance of specific protein. For, e.g. kinases as substrates are ideal system for screening, because there are many well designed chemical libraries already available for identification of kinase inhibitors. Pengo and coworkers identified kinases and phosphatases that

regulate ATG4B activity by siRNA which could be helpful in developing a therapeutic anticancer strategy [77].

3 Structure-based classification of anticancer compounds in plants

Plants for their normal growth and development synthesizes mainly; primary or secondary metabolites. Carbohydrates, proteins and lipids are primary and have direct association with regular ongoing necessary mechanisms in them, whereas, secondary metabolites are biosynthetically driven from the primary metabolites and are involved in functions such as resistance towards disease, protection, species interactions and competition [88]. These compounds on the basis of their biosynthetic origins are classified into three main groups: phenolics, terpenoids and nitrogen/sulfur-containing compounds [54]. They are further classified on the basis of their structure as shown in Table 2 [93]. A few examples of anticancer secondary metabolites in regular use and under clinical trials: vincristine, paclitaxel, homoharringtonine, ingenolmebutate, curcumin, betulinic acid [90]. On a different perspective every year, number of new cytotoxic secondary metabolites are identified and with new sources aiming with innovative solutions against deadly cancer. Thus, bringing modifications in these chemical structures can provide a new-fangled strategy for more specificity in action of anti-cancerous drug development [41, 111].

3.1 Plant tissue culture: a doorway with multiple prospects!

Plant tissue culture is considered as basic technique, as a substitute to vegetative propagation in plants and in various commercial applications. It is an indispensable module in mass multiplication of elites, developing true-to-type plant cultures and in regeneration of genetically engineered novel plants. Basically, these are the culture systems with explants as plant cell, tissues or organs grown on artificial media (offers nutrient for its growth) in vitro. Explant obtained from plant tissues gradually transforms into cell mass that appears to be amorphous and colorless to pale brown, in sterile in vitro condition. Passaging callus on fresh media and growth condition at 25 ± 2 °C helps them to maintain indefinitely and when need, by supplementing growth regulators (auxins/cytokinines) and appropriate growth chamber conditions (16 h light, 8 h dark) can again re-differentiated into a whole plant. Callus in general resembles the non-differentiated meristematic cells. They consists small vacuoles but lack photosynthetic apparatus: chloroplast. By nature, they exist in compact, friable and semi friable

Table 2 Structure-based classification of antitumor compounds

Metabolites	Present in	Characteristic structure	Classification
Phenolic compounds	Plant, fruit, spices, vegetable, and grains	synthesized from shikimate pathways, containing one or more hydroxylated aromatic rings	Flavonoids Stilbenes Phenolic acids
Terpenopids	essential oils, resins, or oleoresins	linear arrangements of isoprene, and their carbon skeletons consist of two or more carbon units	Mono-terpenoids Di-terpenoids Tetra-terpenpids
Nitrogen-containing alkaloids and sulfur-containing compounds			
Alaloids	All Plants	derived from amino acids, and have a nitrogen-atom-containing heterocyclic ring	14 sub-groups based on ring structure: pyrrolidine, pyrrolizidine, piperidine, tropone, quinoline, isoquinoline, acridine, quinolizidine, benzopyrrole, indolizidine, imidazole, purine, quinolizidine, and oxazole
Organosulfur Compounds (OCS)	Allium genus, Brassica genus, sulfur-containing organic compounds	sulfur-containing organic compounds	cycloalliin, thiosulfonates, cysteine alkyl disulfides, glucosinolates, goitrin, and epithionitrile

form. Friable callus on slowly shaken condition can give rise single cell cultures [31].

Plant tissue culture was pioneered by Gottlieb Haberlandt (1902), who entrenched first callus culture in roots or embryo cultures, in the beginning of the twentieth century [21]. The period of 1940s and 1960s led to advancements in tissue culture techniques, studies in details of cell behavior: metabolism, cytology, morphogenesis, nutrition, embryogenesis and pathology; regenerated plants which were free from pathogen, clonal propagation and germplasm storage. Also during this phase (end of 1960s) plant tissue culture was considered to be a promising tool for the study and production of various bioactive compounds as the alliance of various gene-based innovative molecular methodologies were developed and were implicated in callus culture and other plant tissue culture techniques [31, 101].

The true search for natural products as potential anticancer compounds dates back to Ebers papyrus in 1550 BC [101], but scientific period of this search began in 1950s with extensive research, identification and isolation of potent cytotoxic compounds like alkaloid vinca (vinblastine and vincristine) and podophyllotoxins [19, 29]. These technical innovations led United States National Cancer Institute (NCI) to initiate worldwide collection of plants in tropical areas during 1960s which in turn helped in discovery of potential novel cytotoxic drugs like campothecins and taxanes [40]. Unfortunately, in 1982 the program of plant collection was terminated, but the screening program in plants for investigation of new anticancer drug was continued in 1986 at tropical and sub-tropical regions [29].

3.2 Tissue culture approaches for the production of anticancer compounds

As its name suggests, medicinal plants are, more or less, used for almost all the types of medical conditions due to their properties of bearing number of secondary chemical. Plant tissue culture of medicinal plants may be proven as promising strategy for the production of such chemicals [40]. Looking into the strategy in details, it involves cell level production (cell culture), tissue level or organ level (organ culture) productions. Before we discuss about the production, first we should know how micropropagation strategy operates.

Plant tissue culture starts with very basic property of any plant cell, i.e. totipotency which states that any plant cell, if nurtured in suitable environment, can regenerate into a new plant. This includes steps like dedifferentiation and re-differentiation of cells and tissues synthesized. In the process of morphogenesis, plant cell undergoes unorganized mass phase which is known as callus (indirect organogenesis) or a new organ, e.g. roots, stems (direct organogenesis) (see Fig. 3). Callus is further converted into new organ by maintaining supplied auxin to cytokinin ratio. Most techniques of organogenesis end up with plant reproduction or crop improvement. However, in case of callus (and cell suspension) culture and hairy root cultures, secondary metabolites can be harvested systematically, and thus, these techniques can be used for the production of plant secondary metabolites [40]. Here, we describe both the techniques in details with case studies of production of anti-cancer compounds.

3.2.1 Callus and cell suspension culture

Callus culture is generally achieved through culturing plant parts like leaf, cotyledon internode on callus inducing medium (CIM). CIM contains higher level of auxins such as NAA or 2,4-D and low levels of cytokinins. This may result in either friable or compact callus. Compact calli are generally green and used for the organogenesis purpose. Friable calli are used for the cell suspension culture. In cell suspension culture system, cells are maintained in their proliferation state with all the physical and chemical factors associated with the growth. After their maintenance, elicitors such as fungal cell walls, modulators such as plant growth regulators and signaling molecules such as jasmonic acid are added which enhance the secondary metabolism. By this means, production of commercially and medically important secondary metabolites is synthesized in simple and easily cultivable cells [31].

3.2.2 Biotransformation: hairy root culture (a propitious approach)

Plants produce numerous chemicals known as phytochemicals and secondary metabolites. Certainly, these chemicals have applications in mainly pharmaceuticals, food and flavor industries. As far as plant growth and survival are concerned, these chemicals help plant to combat in unusual environmental conditions known as stress. However, these chemicals are synthesized in very minute amount but still are very effective; humans are "hungry" for such chemicals with the goal of commercialization. Thus, attempts have been made to escalate these bioactive chemicals using biotransformation methodology such as hairy root culture [94] technique in plant tissues. In current scenario hairy root culture has evolved as a good alternative for production of secondary metabolites with desirable remodeling in bioactive plant metabolites. Hairy roots are form of differentiated organs developed by infection of *Agrobacterium rhizogenes.* These differentiated organs (roots) displays ability to fabricate and release complex active glycoprotein as well as facilitate plants to produce specialized biomolecules by altering its biosynthetic pathways. The growth of roots is

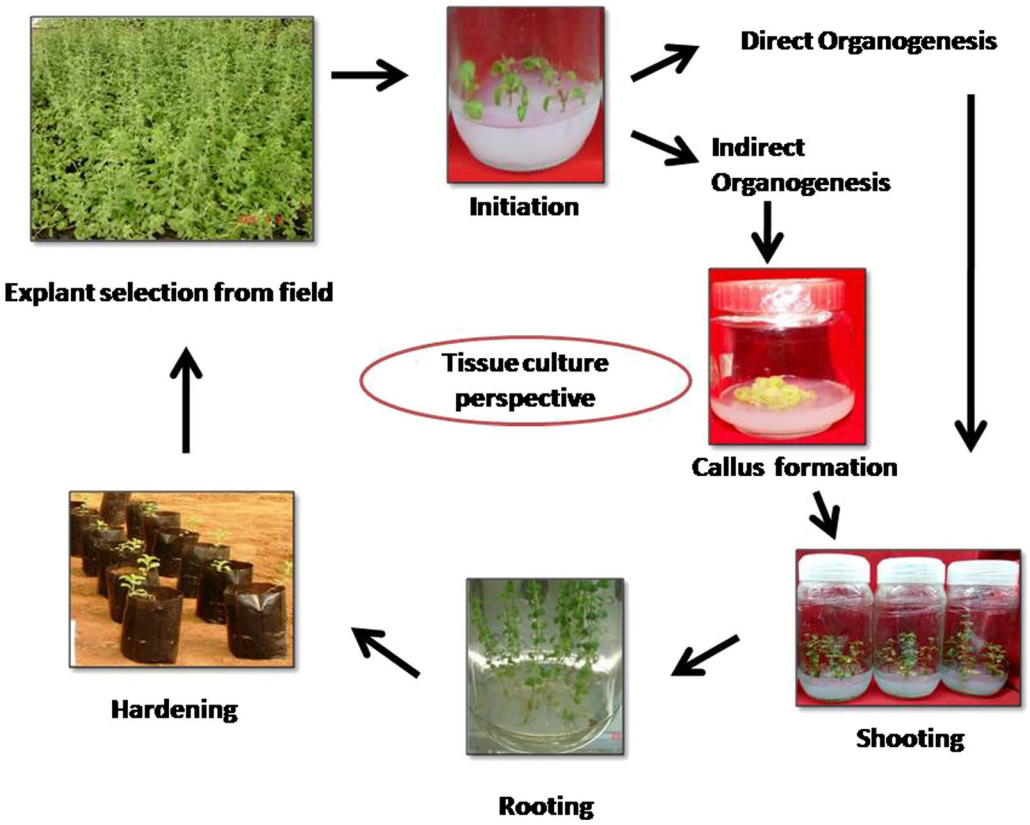

Fig. 3 Overview of plant tissue culture methodology

rapid and vigorous add on to rapid establishment of the hairy root culture. Numerous secondary metabolites can be obtained from these cultures. Molecular mechanism of this is a transformation of genetic material from bacterium to plant cell. *Agrobacterium rhizogenes* contain Ri (Root inducing) plasmid having T-DNA region. These Ri plasmid can be modified and gene of interest can be inserted in the T-DNA region. After infection, bacterium transfers this T-DNA into chromosome of the plant cell. Thus, this technique can be used as a tool for transformation as well as secondary metabolite and its enhanced production inside the roots [52]. As seen in Fig. 4, it starts from explant collection and agro-infection under in vitro conditions which later on results with the formation of hairy roots (highly bulkier than seedling roots). With these roots, some optimization can be carried out such as precursor feeding, elicitation or metabolic engineering to get maximum amount of secondary metabolites.

3.3 Strategies used for production

Biotechnological interventions in field of plant cells and tissues have offered different methodologies such as callus cultures, cell suspension cultures, organ cultures and hairy root cultures (as discussed above) for production of bioactive compounds. The two breakthrough techniques namely: cell cultures and clonal propagation are in wide use. Cell culture study is based on callus initiation via in vitro raised cultures on best suitable medium. Usually such cultures undergo somaclonal variation on frequent sub-culturing. Therefore, callus is first screened and selected (high genetic stability and highly productive cell lines), then taken further for cell suspensions. The final step involves the bioreactor which may probably lead to commercial production of bioactive compound. Transition from shaker flask to bioreactors is the most critical step while scaling up the production [105].

Bioreactors now have become one of the crucial contrivances in cell-based strategic productions. They are functioning as a biological factory to produce bioactive compounds and research from past many years conclude for its variable benefits such as: homogeneity, controlled cultural and physical environment, reproducible yields under controlled growth conditions, better control for scale up, simple and fast harvest of cells, easier separation of targeted compound etc. There are different bioreactor designs made for specific growth conditions for profuse cell growth and suitable secondary metabolite production. Bioreactors types are classified as (1) Stirred

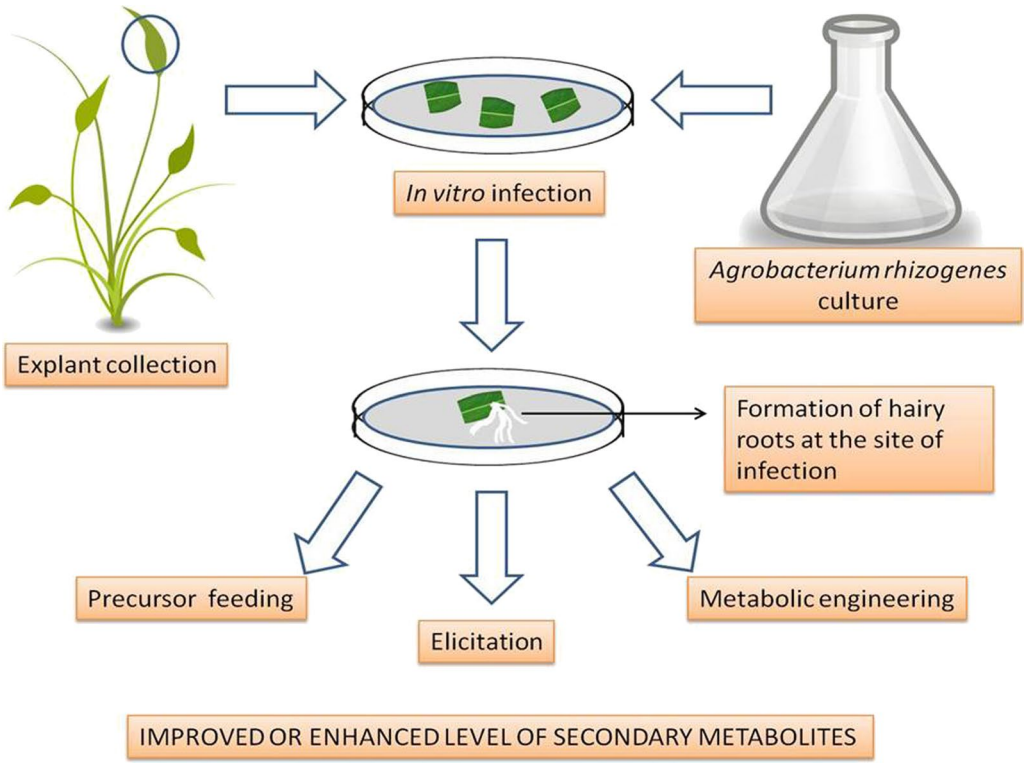

Fig. 4 Hairy root culture technique

tank bioreactor, (2) Airlift bioreactor, (3) Fluidized bed bioreactor, (4) Packed bed bioreactor, (5) Photo-bioreactor and (6) Membrane bioreactor. Scale up strategy in general, starts from lab scale and reproducing it as nearly as possible for production at larger number of products. An archetypal scale-up series in plant cell and tissue culture studies involve initiation with simple jars, to 1 L shake flasks, then 1–10 L glass bioreactors, after which it is scaled up through stainless steel vessels of varying size from 30–150 to 1000 L (see Fig. 5) (Sarkar et al. 2018).

Further to increase production, different viewpoints were used to carry out:

1. Standardization of culture medium: Culture media is one of the important constituent in bioreactor system. Optimal conditions can be found by changing the chemical and physical factors of the culturing system. These factors include chemical components or phyto-hormones in the medium, pH, aeration, temperature, light, addition of antifoams etc. [105].
2. Elicitors or Precursors: Occasionally precursors and elicitors are added in culture system to enhance its production level; however the timing of addition into media and type or nature of precursor chosen is important. *For, e.g.* the study conducted by Sivanandhan and his coworkers concluded that, in bioreactor they obtained 1.66 fold higher concentration of total withanolides as compared to control *Withaniasomnifera* cell suspension culture via addition of elicitors such as: aluminium chloride, chitosan and cadmium chloride; and precursors: cholesterol, mevalonic acid and squalene [96].
3. Cell lines selection: The variation in production entity from cell to cell in a heterogeneous cell mass provides an opportunity in selection of high production strain. Selected strains are normally cloned and plated on agar medium to form colonies. Then, amount of active compound is measured through radioimmunoassay (RIA) or HPLC (High Performance Liquid Chromatography) methods for selecting colony of high production yield. For selection criteria there are chiefly three ways:

   a. *Visual*—It is on the basis of visual marking such as color of product.
   b. *Nutritional requirement or chemical resistance*—The selection by growing cells on a selective media such as specific nutrient deficient or in presence of some chemical inhibitor.

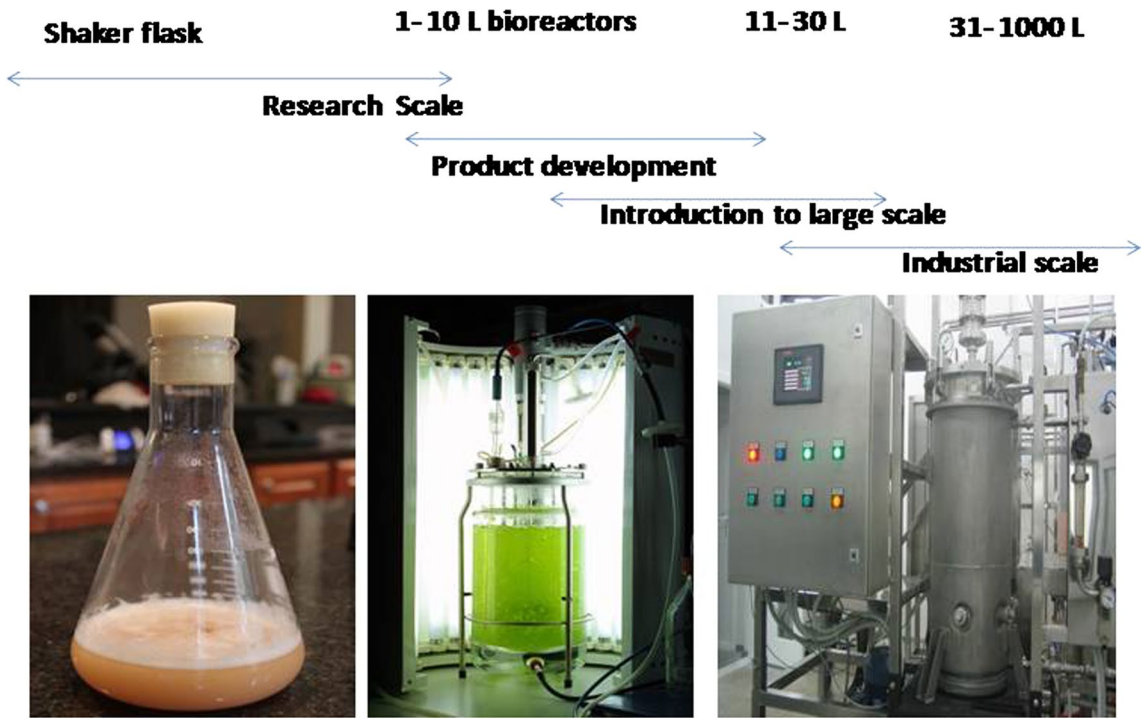

Fig. 5 Scale-up of bioreactors from lab to commercial scale

c. *Production analysis*: cells with high production yield can be selected by developing a rapid, sensitive method to analyze the metabolites.

4. Mutation: Mutagenesis using nitrosoguanidine, radiation (UV, X-ray, or ϒ-ray) or others can sometimes produce the cells to increase the productivity of metabolites, though in plants this method is not easy, since most of the vegetative cells are diploid. Baskaran et al. [18] reported two induced EMS macro-mutants (necrotic leaf and nerium leaf) of periwinkle which result increased content in root and leaf alkaloids and anticancer leaf alkaloids, vincristine and vinblastine than the parental variety. Kannabiran et al. [49] concluded that gamma radiation (40 Kr) and chemical mutagen (EMS-30 mM) increased content by 0.37% and 0.32% respectively.
5. Natural Products Genomics: A novel approach intended to access the plants own genomic capacity to boost yields and transform complex bioactive metabolite. This technology unifies the gain of functional mutagenesis and selection to (a) imitate the development of novel compounds in plants, and (b) to increase yield of known bioactive compounds. And carry out selection at pace during cell culture level in mutants consisting of large population [91].
6. Morphological Variation: There are some evidences describing the role of undifferentiated [68] and differentiated cells in production of secondary metabolites at higher levels.
   The strategy of hairy root culture was reported back in 1985 by Flores and Filner, they introduced hairy root cultures induced by *Agrobacterium rhizogenes* have potential to increase productivity. It is also a useful method to induce the function of the plants to produce the metabolites when the cells grown in a proliferating medium are transferred to a medium for its differentiation. Hanafy et al. [42] showed the production of indole alkaloids (i.e vinblastine, vincristine and catharanthine) in hairy roots cultures of *Catharanthus roseus* L. in leaf explants and stems.
7. Immobilized Cells: It refers to confinement or localization of cells into a defined region. Immobilization helps to combat problems of cell aggregation and low shear resistance. The surface immobilization and cell aggregation technique are in wide use, wherein cells are entrapped in particular gel or combinations of different gels. Generally alginate, carrageenan, agar, agarose, polyacrylamide, etc. are used to immobilize the cells. This technique has several benefits such as: high cell density in small scale bioreactor which are cost efficient and with less risk of contamination, easy

and simple downstream processing and extension of cell viability in stationary phase, higher product accumulation. But still, there is dilemma regarding, extracting out the metabolites from cells and to prevent cell damage by different treatments are need of investigation. There are few cases which reports that plant cells can secrete metabolites exterior to cells in media provided. The initial reports were production of indole alkaloids or digoxin using immobilized cells by [56].

4 Case studies on tissue culture-based production of anticancer compounds

The recent investigations and in-depth knowledge about novel natural structures with important biological activity and mechanisms have remarkable influence on pharmaceutical business. The discoveries of various anticancer compounds in association with plant tissue culture hold excellent new possibilities to tackle the disease 'cancer'. The tissue culture operations used for production of cytotoxic secondary metabolites have potentially led to production of wide variety compounds of like terpenoids, alkaloids, flavanoids, steroids and amino acids. Few successful attempts are discussed here and enlisted in Table 3.

4.1 Taxol (Paclitaxel)

A complex diterpene alkaloid present in the bark of the Taxus tree, is one of the most potent anticancer compounds due to its mode of action on the micro tubular cell system. It is in market, back since 1993 with trade name Taxol® [20]. The two important factors which are noteworthy with increasing demand of taxols are: trees take years to grow and mature, and majorly the taxol concentration in tissues is very less. To overcome these situations, plant tissue culture prompts to be a potential, stable and long-term method to produce taxoids at commercial scale. Taxol production in *Taxus baccata* callus culture was carried out with salicylic acid pretreatment to enhance the resistance against water stress and oxidative stress. One of the most important taxanes, taxol, was also elevated 5.1 times higher as compared to control calli. Total taxanes was increased up to 3.5 fold [89]. Salehi et al. [87] worked out in modifying media as M10 medium (MS medium with pH 6.0 and supplemented

Table 3 Examples of therapeutic plants under plant tissue culture trials with anticancer activity

Plant	Explant used	Culture technique	Bioactive agent	Media	Cancer	References
Parwal (*Andrographis lineate*)	Mature leaf explant	Callus culture	7-*O*-Methylwogonin (MW) and Echioidinin (ED)	MS with 1.0 mgL^{-1} IAA	Leukemic cell line, CEM	[55]
Periwinkle (*Catharanthus roseus*)	Root	Hairy root culture	Vincristine	MS	Cancer	[56]
Turnip (*Brassica rapa)*	Seeds	Hairy root culture	Glucosinolates	DMEM medium supplemented with 10% FBS	Colon HT-29 (human colorectal adenocarcinoma) and oestrogen-dependent breast MCF-7 (human breast adenocarcinoma) cell lines	[78]
Plume poppy (*Macleaya cordata*)	Leaves and stems	Hairy root culture	Sanguinarine	Hormone-free MS media, 200 mg L^{-1} timentin	Colon cancer	[75]
Hedgehog coneflower (*Echinacea purpurea*)	Leaf	Callus culture	Flavanoids	MS,2,4-D, NAA, ZnO nanoparticles	MCF-10 cells and peripheral blood monolayer cells	[76]
Golden flax (*Linum flavum*)	Root	Hairy Root Cultures	Aryltetralin lignin	Gamborg B5 medium with methyl jasmonate, and ferulic acid	Cancer	[82]
Dessert bush *(Aerva javanica)*	Leaf and leaf derived callus	Callus culture	–	MS with IAA and NAA	MCF-7 breast cancer cell line	[83]
Tea (*Camellia sinensis*)	Leaf	Hairy root cultre	Polyphenols	1% MS (Murashige and Skoog) and water	–	[77]
Phyllanthus amarus	Shoot	Hairy root culture	Reactive Oxygen Species	MS with 2.0 mg L^{-1} BAP	MCF-7 cell	[2]

with 1000 mg L^{-1} spirulina powder, 1000 mg l^{-1} casein hydrolysate and 3 g L^{-1} Gelrite) for improved callus growth and improved taxol production (106.6 µg L^{-1}) as compared to control (77.7 µg L^{-1}) in Hazel (*Corylus avellana* L.) plant. Filová and Krivosudská reported Gamborg's B5 medium supplemented with 2,4-dichlorophenoxiacetic acid (2 mg L^{-1}), kinetin (0.5 mg L^{-1}) and gibberellic acid (0.25 mg L^{-1}) for callus growth and elevated taxane production in *Taxusbaccata* L. [36].

4.2 Podophyllotoxin

It is anaryltetralin (2, 7 Cyclolignan) lignin occurring in *Podophyllum peltatum* and *Podophyllum hexandrum*. It also serves as an initial material for preparation of its semisynthetic derivatives, teniposide and etoposide, commonly used in anti-tumor therapy [16]. Slow growth rate of plants and practice of using rare wild plants for healthcare, results in its limited supply against the booming demand. This condition has called for alternative methods for production of podophyllotoxin. First attempt of using tissue culture technique was carried out by Kadkade in 1982 [114] to increase yield of podophyllotoxin. Although it was difficult to enhance this anticancer compound in cell suspension culture, Lalaleo et al. [57] optimized the culture method with respect to organogensis from callus and found maximum level of podophyllotoxins in dark treated culture which again showed morphogenic structures and they established clear relationship between concentration of these metabolites and organogenic capacity. Hence, nurturing the callus mass for organogenic pathway may be recommended to get higher content of podophyllotoxin and its derivatives. Taking an advantage of both the hairy roots and elicitation, Tashackori et al. [98] attempted the gene expression level of secondary metabolites, mainly podophyllotoxin and its derivatives (lignans) using cell wall of *Piriformospora indica* on hairy roots of *Linum album* and found significantly higher expression of genes involved in secondary metabolites such as phenylalanine ammonia-lyase, cinnamyl alcohol dehydrogenase, cinnamoyl-CoA reductase and pinoresinol–lariciresinol reductase. As a result, podophyllotoxin was increased from 64.14 µg G^{-1} of dry roots to 124.46 µg G^{-1} (1.94 times) in 1% v/v concentration of elicitor.

4.3 Vincristine and vinblastine

These compounds are dimeric indole alkaloids obtained from *Catharanthus roseus.* They have great antitumour activity against various solid tumors and leukemias. Commercially, the procurement of these compounds from *Catharanthus roseus* consumes large quantity of these plants and also the intact plant contains low concentrations of these compounds, about 0.0005%. Alternative strategy pioneered is plant tissue culture. Mishra and coworkers established a culture system in *Catharanthus roseus* with B5 medium, in addition with 3% sucrose, 0.5 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg L^{-1} Kinetin (KIN), 2 mg L^{-1} α-naphthalene acetic acid (NAA) and obtained enhanced alkaloid production by 5.67 mg G^{-1} dry weight [102]. Also the addition of elicitor "chitosan" leads to enhanced production of vincristine and vinblastin in cell culture of *Catharanthus roseus* (L.) G. Don as studied by Pliakong and their coworkers. They reported the highest amount of accumulation of vincristine and vinblastin at 4.15 and 5.48 µg Mg^{-1} cell dry weights respectively in cell suspension culture at 14th day [65].

4.4 Camptothecin

It is a cytotoxic quinone alkaloid which inhibits enzyme DNA topoisomerase I and is isolated from bark and stem of *Camptotheca acuminate* (Happy tree) [80] and is used as anticancer drug worldwide. Lower yield of camptothecin from whole plant, poor seed germination and deficit viable method for production has been opted for tissue culture for its production through various available plants like *Merriliodendron megacarpum, Eravatamia heyneana, Nothapodytes foetida and Ophiorrhiza acuminata, to* meet the enormous demand of camptothecin [102]. Attempts were also carried out by Wetterauer et al. [109] to produce camptothecin from *Ophiorrhizasa mungos* through infection of *Agrobacterium rhizogenes*. They cultured 25 different lines of *Ophiorrhiza mungos* to produce hairy roots and found maximum of 3.2 mg of camptothecin/g of dry weight. With their experiments, they made this production feasible and scalable so that it can be used by pharmaceutical industries. Deepti and Sateeshkumar [30] reported enhanced production of camptothecin in cell suspension cultures of *Ophiorrhiza mungos* Linn. through elicitor (silver nitrate and yeast extract (YE) treatment. About 13.3-fold enhanced production and threefold increments in cell growth was obtained in cell cultures elicited with 50 mg L^{-1} YE on 10th day.

4.5 Curcumin

Curcumin, a part of polyphenol superfamily a bioactive component of turmeric (an Indian spice-obtained from dried rhizomes of *Curcuma longa* plant) shows anticancer activity [104], via inhibition of cancer cell proliferation, metastasis, induction of cell cycle arrest and apoptosis. The rhizomes of turmeric were studied for their anticancer activity in vitro using tissue culture

Table 4 Potential bioactive compounds targeted with anticancer properties in plants

Plant species	Common name	Active compound	Explant	Cancer cell lines	References
Carica Papaya	Papaya	Polyphenols	Seeds	Prostate cancer (PC-3) Cell line	[10]
			Aqueous leaf extract	Breast Cancer Cells MCF-7	[115]
Smilax China	Madhusnuhi	Kandelin B-5 (1)	Rhizome	Acute monocytic leukemia (THP-1) cells	[113]
Achyranthes aspera	Devil's horsewhip/ Prickly Chaff flower	Steroidal saponins, phenolic acids, alkaloids,	Root extract	Liver (Hep-2) & colon (HT-29) cell line	[95]
Syzygium aromaticum	Clove	Eugenin	Dried buds powdered cloves extract	HT-29 colon cancer cells	[60]
Citrus Peel	–	Polymethoxy-flavones	Juice	Blocks the metastasis cascade, inhibit cancer cell mobility in circulatory systems, proapoptosis, and antiangiogenesis	[107]
Cinnamomum burmannii	Cinnamon	Cinnamic acid	Cisplatin and Cinnamon essential oil	HeLa cells	[58]
		Trans-cinnamaldehyde	Cinnamon Oil	MDA-MB-231 breast cancer cell line	[100]
Mangifera indica L	Mango	Antioxidants	Kernal	Human breast cancer (MCF-7 and MDA-MB-231 cell lines	[1]
Metha spp. (Mentha arvensis, M. longifolia, M. spicata and M. viridis)	Mint	Phenols and flavonoids	Whole plant extract	A-549, COLO-205, HCT-116, MCF-7, NCI-H322, PC-3, THP-1 and U-87MG from six different origins (breast, colon, glioblastoma, lung, leukemia and prostate cell lines	[92]
Mentha longifolia			Leaves extract	HepG2 and Vero mammalian cell lines	[6]
Zingiber officinale	Ginger	Quercitin	Leaves	HCT116, SW480 and LoVo human colorectal cell lines	[74]
			Rhizome	human cervical cancer HeLa cells and breast cancer MDA-MB-231 cell	[14]
Ficus carica	Fig	Flavonoids and polyphenols	Fruit	MCF 7 breast cancer cell lines	[46]
Cinnamomum verum	Cinnamon	Cinnamic acid	Essential oil	(FaDu, Detroit-562 and SCC-25) head and neck carcinoma cells	[110]
Moringa oleifera	Drum sticks	Eugenol, isopropyl isothiocynate, D-allose, and hexadeconoic acid ethyl ester	Leaves, bark and seed extract	MDA-MB-231 and HCT-8 Breast and colorectal cancer cell lines	[8]
Colochirus anceps	Sea cucumber	Triterpene glycosides		Human leukemia and colorectal cancer cells	[13]
Coffea Arabica and coffee robusta	Coffee	Caffeine, Chlorogenic acid, caffeic acid	Coffee beans extract	HeLa and PA-1 cell lines	[82]
Coffea arabica				HT-29 (colon) and SCC-25 (oral) cancer cell lines	[69]
Hypericum perforatum	St Johns Wort	Hypericin	Aerial part	MCF-7 breast cancer cell line	[64]
Morus nigra	Mulberry	Resveratrol (stillbene – polyphenol)	Fruit extract	Breast cancer cell line (MCF 7)	[4]
Argemone mexicana L	Prickly poppy	Beberine	Stem and leaves	A549, SiHa and KB Cell lines	[70]
Taraxacum	Dandelion	Flavonoid	Root extract	HT-29 colorectal cell line	[73]

Table 4 (continued)

Plant species	Common name	Active compound	Explant	Cancer cell lines	References
Glycine max	Soybean	Isoflavones and saponins (Daidzein)	Seeds	HT29 colon cancer cell line	[59]
Origanum vulgare L	Oregano	Carvacrol and thymol	Aerial part	Hepatocarcinoma HepG2 cancer cell line	[33]
Piper nigrum	Black pepper	Piperine	Seeds	Colorectal carcinoma cell lines (HCT-116, HCT-15, and HT-29)	[78]
Capsaic annum L	Red chilli	Capsaicin	Fruit	HepG2 hepatocellular cancer cells	[5]
Gossypium barbadense L	Cotton	Phenolics	Leaves	Bone Cord Cells	[72]
Annona muricata L	Custard apple	Annonacin	Leaf extract	MCF-7 cell	[86]
Avena sativa	Oat	β-glucan	oats β-glucan	Me45 melanoma cell line	[25]
Curcuma longa	Turmeric	Curcumin	Tuber	Her2 breast cancer cell lines	[61]
Daphne striata Tratt	-	Mezerein	Aerial parts	MCF-7, A549, LNCaP, ACHN, and C32	[103]
D. mezereum L					
Camellia Sinensis	Tea	Flavonoids and polyphenol	Young leaves	Caco-2, colon carcinoma cells	[35]
Vitis venifera	Grapes	Oligomeric proanthocyanidins	seed extract	H716 colorectal cancer cells	[83]
Allium cepa	Onion	Flavonoids (Fisetin)	Onion bulb	MDA-MB-231 breast cancer cell line	[38]
Rubus idaeus L	Wild Raspberry	Flavonoids and phenolics	Leaf extract	HCT-116 colorectal cell line	[106]
Matricaria recutita L	Chamomile	terpenoids α-bisabolol, flavonoids	Flowers	HepG2 Human hepatoma cells	[9]
Olea europaea	Olive	*oleuropein*	Oil	*143B,* Osteosarcoma Cells	[79]

methods, showed cytotoxic effect on Chinese Hamster Ovary (CHO) cells at a concentration of 0.4 $mgML^{-1}$ [55].

4.6 Other promising anticancer compounds

The novel findings in research have brought forth many potential and promising compounds against cancer. Such as sanguinarine [43], flavanoids [50], polyphenols [7], glucosinolates [26], quercitin [14], caffeine, chlorogenic acid, caffeic acid [69, 82] etc. with details are mentioned in Table 3.

A huge amount of research is also targeted on plants and plant-derived natural products. With recent investigations, new plant species with potent anticancer activity either in vivo or in vitro, have added to our knowledge. Few examples plant species with promising anti-cancer activity are: *Achyranthes aspera*, *Allium sativum, Andrographis paniculata, Annona muricata, Bidens pilosa, Astralagus hedysarum, Bolbostemma paniculatum, Cannabis sativa, Centaureaainetensis,Camellia sinensis, Gossypium hirsutum, Daphne mezereum, Mangifera indica, Hypericumper foratum, Nerviliafordii, Hydrocotyle asiatica, Oroxylum indicum, Salvia miltiorrhiza, Scutellaria, Rubia cordifolia, Picrorrhiza kurroa, Silybum marianum, Smilax china, Withania somnifera, Strychnos nuxvomica, Zingiber officinale, Taraxacum officinale* etc. (Table 4).

5 Advantages of plant tissue culture technique in anticancer compound production

The evident role of plant tissue culture in production and identification of novel active anticancer compounds has performed excellent over these past years. As there are many difficulties in procuring these compounds in bulk quantities directly from plants. The practises using callus culture, cell suspension culture and hairy root system has eminent advantages over whole plant cultivation, enlisted below:

1. The targeted compound can be obtained in regulator free media, are easily culturable with less growth time also with more stability in genotypic and biochemical level.
2. The cultured cells within system would be free of microbes, thus contamination rate and losses are minimized.

3. The cells of any plant (rare or endangered species) can easily be managed to produce secondary metabolites as a continuous source, once a system is defined.
4. It is easier to study the active metabolites from cells with known production system and also manage it through robotics, leading to reduced cost and improved productivity.

6 Conclusions

The recent advances and developments of research have given various scopes in fighting cancer. Most reliable and effective sources were found to be natural bioactive compounds. There are various novel compounds actively being discovered such as taxol, camptothecin, vinblastin and vincristin etc. But the availability and amount of compound procurement has always been the question. The progress in bio-techniques, particularly in area of tissue culture has satisfied the question of daily requirement and its availability for the burning demand of population and industrial commencement. The use of plant tissue culture strategy has enhanced a progressive channel for reliable and continuous source of these compounds. Further the insight into the biosynthetic pathways of important anticancer compounds in plants as well as cultures are still unknown, and henceforth these applications are in demand to unravel information grounded at cellular and molecular level to bring forth at industrial level.

Acknowledgements
I would like to show gratitude to my mentor and colleagues in the Department of Plant Tissue Culture and Biotechnology, Anand Agricultural University.

Authors' contributions
PP, VP, AM contributed in writing section of the review, while SK and YMS managed the reviewing and editing portion. All authors read and approved the final manuscript.

Funding
No funding was received for this work.

Availability of data and materials
No additional data and material other than the manuscript is to be produced.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Agricultural Biotechnology, Anand Agricultural University, Anand, Gujarat, India. [2]Rutgers University, New Brunswick, NJ, USA. [3]Agricultural Research Organization, Volcani Center, Rishon LeZion, Israel. [4]College of Agriculture, Anand Agricultural University, Vaso, Gujarat, India.

Received: 28 April 2021 Accepted: 25 January 2022
Published online: 26 March 2022

References

1. Abdullah ASH, Mohammed AS, Abdullah R, Mirghani MES, Qubaisi MA (2014) Cytotoxic effects of *Mangifera indica* L. kernel extract on human breast cancer (MCF-7 and MDA-MB-231 cell lines) and bioactive constituents in the crude extract. BMC Complement Altern Med 14:199
2. Abhyankar G, Suprasannab P, Pandey BN, Mishra KP, Rao KV, Reddy VD (2010) Hairy root extract of Phyllanthus amarus induces apoptotic cell death in human breast cancer cells. Innov Food Sci Emerg Technol 11:526–532
3. Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, Busselberg D (2019) Flavonoids in cancer and apoptosis. Cancers 11(1):28
4. Ahmed A, Ali M, El-Kholie E, El-Garawani I, Sherif N (2016) Anticancer activity of *Morus nigra* on human breast cancer cell line (MCF-7): the role of fresh and dry fruit extracts. J Biosci Appl Res 2(6):352–361
5. Ahmed SS, Faten ZM, Al-shimaa MA (2017) In vitro studies on anticancer activity of capsaicin a component of hot chili pepper against human hepatocellular carcinoma cells. Int J Cell Sci Mol Biol 2(4):555591.
6. Al-Alim KH, Abdelrazik M, Aziz AA, Diab AA, El-Beshbishy HA (2014) Antimutagenic and anticancer activity of Al Madinah Alhasawy Mint (*Mentha longifolia*) leaves extract. Pak J Biological Sci 17(12):1231–1236. https://doi.org/10.3923/pjbs.2014.1231.1236
7. Alagarsamy K, Shamala LF, Wei S (2018) Protocol: High-efficiency in-planta Agrobacterium-mediated transgenic hairy root induction of *Camellia sinensis* var. sinensis. Plant Methods 14:17
8. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani HA, Islam M (2015) Moringa oleifera as an anti-cancer agent against breast and colorectal cancer cell lines. PLoS ONE 10(8):e0135814
9. Al-Dabbagh B, Elhaty IA, Elhaw M, Murali C, Al-Mansoori A, Awad B, Amin A (2019) Antioxidant and anticancer activities of chamomile (*Matricaria recutita* L.). BMC Res Notes 12:3
10. Alotaibi KS, Li H, Rafi R, Siddiqui RA (2015) Papaya black seeds have beneficial anticancer effects on PC-3 prostate cancer cells. J Cancer Metastasis Treat 3:161–168
11. Al-Sokari SS, El-Sheikha AF (2015) In vitro antimicrobial activity of crude extracts of some medicinal plants from Al-Baha region in Saudi Arabia. J Food Nutr Sci 3(1–2):74–78
12. Amaral RG, Santos SA, Andrade LN, Severino P, Carvalho AA (2019) Natural products as treatment against cancer: a historical and current vision. Clin Oncol 4:1562
13. Aminin DL, Menchinskaya ES, Pisliagin EA, Silchenko AS, Avilov SA, Kalinin VI (2015) Anticancer activity of sea cucumber triterpene glycosides. Mar Drugs 13:1202–1223
14. Ansari JA, Ahmad MK, Khan AR, Fatima N, Khan HJ, Rastogi N, Mishra DP, Mahdi AA (2016) Anticancer and antioxidant activity of *Zingiber officinale* Roscoe rhizome. Indian J ExpBiol 54:767–773
15. Antolak H, Kregiel D (2017) Food preservatives from plants. In: Karunaratne N (ed) Food additives. Innpotech Open Publishing, pp 45–71
16. Ardalani H, Avan A, Ghayour-Mobarhan M (2017) Podophyllotoxin: a novel potential natural anticancer agent. Avicenna J Phytomed 7(4):285–294
17. Balandrin MJ, Klocke JA (1988) Medicinal, aromatic and industrial materials from plants. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Medicinal and aromatic plant. Springer, Berlin, pp 1–36
18. Baskaran K, Srinivas K, Kulkarni RN (2013) Two induced macro-mutants of periwinkle with enhanced contents of leaf and root alkaloids and their inheritance. Ind Crops Prod 43:701–703
19. Bedi A, Adholeya A, Deshmukh SK (2017) Novel anticancer compounds from endophytic fungi. Curr Biotechnol 6(1):1–17

20. Bernabeu E, Cagel M, Lagomarsino E, Moretton M, Chiappetta DA (2017) Paclitaxel: what has been done and the challenges remain ahead. Int J Pharm 526:474–495
21. Bonner J (1936) Plant tissue cultures from a hormone point of view. Proc Natl Acad Sci USA 22(6):426–430
22. Boy HIA, Rutilla AJH, Santos KA, Ty AMT, Yu AI, Mahboob T et al (2018) Recommended medicinal plants as source of natural products: a review. Digital Chin Med 1:131–142
23. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424
24. Chan HK, Ismail S (2014) Side effects of chemotherapy among cancer patients in a Malaysian general hospital: experiences, perceptions and informational needs from clinical pharmacists. Asian Pac J Cancer Prev 15(13):5305–5309
25. Choromaska A, Kulbacka J, Harasym J, Dubiska-magiera A, Saczko J (2017) Anticancer activity of oat β-glucan in combination with electroporation on human cancer cells. Acta Polon Pharma Drug Res 74(2):616–623
26. Chung IM, Kaliyaperumal R, Rajakumar G, Thiruvengadam M (2016) Production of glucosinolates, phenolic compounds and associated gene expression profiles of hairy root cultures in turnip (*Brassica rapa* sp. *rapa*). 3 Biotech 6(2):175
27. Clinical Trials .gov: National Library of Medicine (US), 2017. https://www.nia.nih.gov/health/ what are clinical trials and studies.
28. Dabrowska A, Thaul S (2018) How FDA approves drugs and regulates their safety and effectiveness. Congress Res Serv 7–5700:1–25
29. Dar KB, Bhat AH, Amin S, Anees S, Zargar MA, Masood A, Ganie SA (2017) Herbal compounds as potential anticancer therapeutics: current. Ann Pharmacol Pharm 2(20):1106
30. Deepthi S, Satheeshkumar K (2016) Enhanced camptothecin production induced by elicitors in the cell suspension cultures of *Ophiorrhiza mungos* Linn. Plant Cell Tiss Organ Cult 124:483–493. https://doi.org/10.1007/s11240-015-0908-y
31. Efferth T (2019) Biotechnology applications of plant callus cultures. Engineering 5:50–59
32. Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmocol 4(177):177
33. Elshafie HS, Armentano MF, Carmosino M, Bufo SA, Feo VD, Camele I (2017) Cytotoxic activity of *Origanum vulgare* L. on hepatocellular carcinoma cell line HepG2 and evaluation of its biological activity. Molecules 22:1435
34. El-Sheikha AF (2017) Medicinal Plants: ethno-uses to biotechnology era. In: Malik S (ed) Biotechnology and production of anticancer compounds. Springer, Berlin, pp 1–38
35. Esghaei M, Ghaffari H, Rahimi EB, Ebrahimi TZ, Bokharaei SF, Motevalian M (2018) Evaluation of anticancer activity of *Camellia sinensis* in the caco-2 colorectal cancer cell line. Asian Pac J Cancer Prev 19(6):1697–1701
36. Filova A, Krivosudska E (2017) Improved taxanes production in callus cultures of *Taxus baccata* L. Res J AgriSci 49(1):1–11
37. Flores HE, Filner P (1985) Metabolic relationships of putrescine, GABA and alkaloids in cell and root cultures of Solanaceae. In: Neumann KH, Barz W, Reinhard E (eds) Primary and secondary metabolism of plant cell culture. Springer, Berlin, pp 174–185
38. Fragis M, Murayyan AI, Neethirajan S (2018) Cytotoxic activity and anticancer potential of Ontario grown onion extracts against breast cancer cell lines. Funct Foods Health Dis 8(3):159–174
39. Gharelo RS, Oliaei ED, Bandehagh A, Khodadadi E, Noparvar PM (2016) Production of therapeutic proteins through plant tissue and cell culture. J Biosci Biotechnol 5(1):93–104
40. Gonclaves S, Romano A (2018) Production of plant secondary metabolites by using biotechnological tools. In: Ramasamy VK (ed) Secondary metabolites—sources and applications. Innpotech Open Publishing, London, pp 81–99
41. Guo Z (2017) The modification of natural products for medical use. Acta Pharm Sin B 7:119–136
42. Hanafy M, Matter M, Rady MR (2017) Production of indole alkaloids in *Catharanthus roseus* L. hairy root cultures. In: Malik S (ed) Production of plant derived natural compounds through hairy root culture. Springer, Berlin. https://doi.org/10.1007/978-3-319-69769-7_5
43. Haung P, Xia L, Liu W (2018) Hairy root induction and benzylisoquinoline alkaloid production in *Macleaya cordata*. Sci Rep 8:11986
44. Holland T, Sack M, Rademacher T, Schmale K, Altmann F, Stadlmann J, Fischer R, Hellwig S (2010) Optimal nitrogen supply as a key to increased and sustained production of a monoclonal full size antibody in BY 2 suspension culture. Biotechnol Bioeng 107(2):278–289
45. Hussain A, Qarshi I A, Nazir H, Ullah I (2012) Plant tissue culture: current status and opportunities. Recent advances in plant in vitro culture 1-28.
46. Jasmine R, Manikandan K, Karthikeyan K (2015) Evaluating the antioxidant and anticancer activity of *Ficus carica* fruits. Afr J Biotech 14(7):634–641
47. Kaçar YA, Faber B (2012) Micropropagation of banana. In: Loyola-Vargas VM, Ochoa-Alejo N (eds) Plant cell culture protocols. Humana Press, Totowa, NJ, pp 143–151
48. Kamalanathan D, Natarajan D (2018) Anticancer potential of leaf and leaf-derived callus extracts of *Aerva javanica* against MCF-7 breast cancer cell line. J Cancer Res Ther 14(2):321–327. https://doi.org/10.4103/0973-1482.171210
49. Kannabiran K, Rajamani K, Suresh J, Joel JR, Uma D (2017) Variability in alkaloid content and phytochemical profile of periwinkle (*Catharanthus roseus* L.) cv. Local through gamma and ethyl methane sulphonate. J Pharmacogn Phytochem 6(5):2528–2532
50. Karimi N, Behbahani M, Dini G, Razmjou A (2018) Enhancing the secondary metabolite and anticancer activity of *Echinacea purpurea* callus extracts by treatment with biosynthesized ZnO nanoparticles. Adv Nat Sci Nanosci Nanotechnol 9:045009
51. Khan T, Gurav P (2017) PhytoNanotechnology: enhancing delivery of plant based anti-cancer drugs. Front Pharmacol 8:1002
52. Khatodia S, Biswas KA (2014) Comparative study of hairy root culture induction efficiency in four medicinally important plants using *Agrobacterium rhizogenes*. Int J Curr Microbiol Appl Sci 3(5):625–633
53. Kumar S, Bajaj S, Bodla RB (2016) Preclinical screening methods in cancer. Indian J Pharmacol 48(5):481–486
54. Kumar A, Irchhaiya R, Yadav A, Gupta N, Kumar S, Gupta N et al (2015) Metabolites in plants and its classification. World J Pharm Sci 2015(4):287–305
55. Kuttan R, Bhanumathy P, Nirmala K, George MC (1985) Potential anticancer activity of turmeric (*Curcuma longa*). Cancer Lett 29:197–202
56. Lakshmanan G, Bupesh G, Vignesh A, Sathiyaseelan A, Murugesan K (2016) Micropropagation and anticancer activity of methanolic extract of *Plumbago auriculata* Lam. Int J Adv Biotechnol Res 7(4):2001–2011
57. Lalaleo L, Testillano P, Risueno MC, Cusido RM, Palazon J, Alcazar R, Bonfill M (2018) Effect of *in vitro* morphogenesis on the production of podophyllotoxin derivatives in callus cultures of Linum album. J Plant Physiol 228:47–58
58. Larasati YA, Putri DDP, Utomo RY, Hermawan A, Meiyanto E (2014) Combination of cisplatin and cinnamon essential oil inhibits HeLa Cells proliferation through cell cycle arrest. J Appl Pharm Sci 4(12):014019
59. Lima A, Oliveira J, Saude F, Mota J, Ferreira RB (2017) Proteins in Soy might have a higher role in cancer prevention than previously expected: soybean protein fractions are more effective mmp-9 inhibitors than non-protein fractions, even in cooked seeds. Nutrients 9(201):1–16
60. Liu H, Schmitz JC, Wei J, Cao S, Beumer JH, Strychor S, Cheng L, Liu M, Wang C, Wu N, Zhao X, Zhang Y, Liao J, Chu E, Lin X (2014) Clove extract inhibits tumor growth and promotes cell cycle arrest and apoptosis. Oncol Res 21(5):247–259
61. Liu HT, Ho YS (2018) Anticancer effect of curcumin on breast cancer and stem cells. Food Sci Human Wellness 2:134–137
62. Market Research Future (MRFR). Herbal medicine market research report—global forecast till 2023. MarketResearchFuture.com. 2018.
63. Mazri MA, Meziani R (2015) Micropropagation of date palm: a review. Cell Dev Biol 4(3):160
64. Mirmalek SA, Azizi MA, Jangholi E, Yadollah-Damavandi S, Javidi MA, Parsa Y et al (2016) Cytotoxic and apoptogenic effect of hypericin, the bioactive component of *Hypericum perforatum* on the MCF-7 human breast cancer cell line. Cancer Cell Int 16:3
65. Mishra MR, Srivastava RK, Akhtar N (2018) Enhanced alkaloid production from cell culture system of *Catharanthus roseus* in combined effect

of nutrient salts, sucrose and plant growth regulators. J Biotechnol Biomed Sci 1(4):14–34
66. Mishra M, Shukla N, Chandra R (2007) Micropropagation of papaya (*Carica papaya* L.). In: Mohan Jain S, Häggman H (eds) Protocols for micropropagation of woody trees and fruits. Springer, Dordrecht, pp 437–441
67. Modi AR, Patil G, Kumar N, Singh AS, Subhash N (2012) A simple and efficient in vitro mass multiplication procedure for *Stevia rebaudiana* Bertoni and analysis of genetic fidelity of in vitro raised plants through RAPD. Sugar Tech 14(4):391–397
68. Mohammed A, Chiruvella KK, Rao YK, Geethangili M, Raghavan SC, Ghanta RG (2015) In vitro production of Echioidinin, 7-O-Methywogonin from callus cultures of *Andrographislineata* and their cytotoxicity on cancer cells. PLoS ONE 10(10):e0141154
69. Mojica BE, Fong LE, Biju D, Muharram A, Davis IM, Vela KO et al (2018) The impact of the roast levels of coffee extracts on their potential anticancer activities. J Food Sci 83(4):1125–1130
70. More NV, Kharat AS (2016) Antifungal and anticancer potential of *Argemone mexicana* L. Med 3:28
71. NCI-60 Human tumor cell lines screen dtp.cancer.gov: National Cancer Institute; 2015. https://dtp.cancer.gov/discovery_development/nci-60/.
72. Oliveira TDD, Rodrigues JM, Miguel TS, Anjos NKC, Abreu JC, Rocha LLV, Dias RXL, Costa DA (2017) Evaluation of cytotoxicity of *Gossypium barbadense* L. extract in bone cord cells through the micronucleus test. J Biosci and Med 5(7):84–91
73. Ovadje P, Ammar S, Guerrero JA, Arnason JT, Pandey S (2016) Dandelion root extract affects colorectal cancer proliferation and survival through the activation of multiple death signalling pathways. Oncotarget 7(45):73080–73100
74. Park GH, Park JH, Song HM, Eo HJ, Kim MK, Lee JW, Lee MH, Cho KH, Lee JR, Cho HJ, Jeong J, B. (2014) Anticancer activity of ginger (*Zingiber officinale*) leaf through the expression of activating transcription factor 3 in human colorectal cancer cells. BMC Complement Altern Med 23(14):408
75. Parsons J, Wirth S, Dominguez M, Bravo-Almonacid F, Giulietti AM, Talou JR (2010) Production of human epidermal growth factor (hEGF) by in vitro cultures of *Nicotiana tabacum*: effect of tissue differentiation and sodium nitroprusside addition. Int J BiotechnolBiochem 6:131–138
76. Pastor M, Pousa AL, Barco E, Segura PP, Astorga GB, Castelo B et al (2018) SEOM clinical guideline in nasopharynx cancer (2017). Clin Transl Oncol 20(1):84–88
77. Pengo N, Prak K, Costa JR, Luft C, Agrotis A, Freeman J, Gewinner CA, Chan AWE, Selwood DL, Kriston-Vizi J, Ketteler R (2018) Identification of kinases and phosphatases that regulate ATG4B activity by siRNA and small molecule screening in cells. Front Cell Dev Biol l 6:148
78. Prashant A, Rangaswamy C, Yadav AK, Reddy V, Sowmya MN, Madhunapantula S (2017) In vitro anticancer activity of ethanolic extracts of *Piper nigrum* against colorectal carcinoma cell lines. Int J Appl Basic Med Res 7:67–72
79. Przychodzen P, Wyszkowska R, Gorzynik-Debicka M, Kostrzewa T, Kuban-Jankowska A, Gorska-Ponikowska M (2019) Anticancer potential of Oleuropein, the polyphenol of olive oil, with 2-methoxyestradiol, separately or in combination, in human osteosarcoma cells. Int Inst Anticancer Res 39(3):1243–1251
80. Rai V, Tandon PK, Khatoon S (2014) Effect of chromium on antioxidant potential of *Catharanthus roseus* varieties and production of their anticancer alkaloids: vincristine and vinblastine. BioMed Res Int 934182:1–10
81. Rao RS, Ravishankar GA (2002) Plant cell cultures: chemical factories of secondary metabolites. Bio-technol Adv 20:101–153
82. Rao S, Nadumane VK (2015) Evaluation of the anticancer potential of coffee beans: an in vitro study. Indian J Trad Knowl 15(2):266–271
83. Ravindranathan P, Pasham D, Goel A (2019) Oligomericproanthocyanidins (OPCs) from grape seed extract suppress the activity of ABC transporters in overcoming chemoresistance in colorectal cancer cells. Carcinogenesis 40:412–421
84. Renouard S, Corbin C, Drouet S, Medvedec B, Doussot J, Colas C et al (2018) Investigation of *Linum flavum* (L.) hairy root cultures for the production of anticancer aryltetralin lignans. Int J Mol Sci 19(990):1–18
85. Richter LJ, Thanavala Y, Arntzen CJ, Mason HS (2000) Production of hepatitis B surface antigen in transgenic plants for oral immunization. Nat Biotechnol 18(11):1167–1171
86. Roduan MRM, Hamid RA, Sulaiman H, Mohtarrudin N (2017) *Annona muricata* leaves extracts prevent DMBA/TPA-induced skin tumorigenesis via modulating antioxidants enzymes system in ICR mice. Biomed Pharmacother 94:481–488
87. Salehi M, Moieni A, Safaie N (2017) A novel medium for enhancing callus growth of Hazel (*Corylus avellana* L.). Sci Rep 7(1):15598
88. Santhi KS, Sengottuvel R (2016) Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int J Curr Microbiol Appl Sci 5:633–640
89. Sarmadi M, Karimi N, Palazón J, Ghassempour A, Mirjalili MH (2018) The effects of salicylic acid and glucose on biochemical traits and taxane production in a *Taxus baccata* callus culture. Plant Physiol Biochem 132:271–280
90. Seca AML, Pinto DCGA (2018) Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. Int J Mol Sci 19:263
91. Sevimli-Gur C, Cetin B, Akay S, Gulce-Iz S, Yesil-Celiktas O (2013) Extracts from black carrot tissue culture as potent anticancer agents. Plant Foods Hum Nutr 68(3):293–298
92. Sharma V, Hussain S, Gupta M, Saxena AK (2014) In vitro anticancer activity of extracts of Mentha Spp. against human cancer cells. Indian J Biochem Biophys 51(5):416–419
93. Shin S, Moon SY, Kim WY, Paek SM, Park HH, Lee CS (2018) Structure-based classification and anti-cancer effects of plant metabolites. Int J Mol Sci 19:2651
94. Singh R, Chattopadhyay T, Thakur D, Kumar N, Kumar T, Kumar SP. Hairy root culture for in vitro production of secondary metabolites. In Kumar N. A promising biotechnological approach: conservation, genetic improvement and utilization. 2018. p. 235-50. https://doi.org/10.1007/978-981-13-0535-1_10.
95. Singh S, Verma SK, Singh SK (2017) In vitro anticancer activity of *Achyranthes aspera* root extract against different human cancer cell lines. Biolife 5(1):119–122
96. Sivanandhan G, Selvaraj N, Ganapathi A, Manickavasagam M (2014) Enhanced biosynthesis of withanolides by elicitation and precursor feeding in cell suspension culture of *Withania somnifera* (L) Dunal in shake-flask culture and bioreactor. PLoS ONE 9(8):e104005
97. Sultan AS, Mohammed FZ, Abbas AM (2017) In vitro studies on anticancer activity of capsaicin a component of hot chili pepper against human hepatocellular carcinoma cells. Int J Cell Sci Mol Biol. https://doi.org/10.19080/IJCSMB.2017.02.555591
98. Tashackori H, Sharifi M, Chashmi NA, Behmanesh M, Safaie N (2018) *Piriformospora indica* cell wall modulates gene expression and metabolite profile in Linum album hairy roots. Planta 248(5):1289–1306
99. Taskin-Tok T, Gowder S (2014) Anticancer drug-friend or foe. In: Gowder SJT (ed) Pharmacology and therapeutics. Innpotech Open Publishing, pp 255–269
100. Thompson M, Schmelz EM, Bickford L (2019) Anticancer properties of cinnamon oil and its active component, trans-cinnamaldehyde. J Nutr Food Sci 9:1
101. Thorpe TA (2007) History of plant tissue culture. Mol Biotechnol 37(2):169–180
102. Thriveni HN, Gudasalamani R, Vasudeva R, Ganeshaiah KN, Uma Shaanker UR (2015) Camptothecine and methoxycamptothecine from callus cultures of *Miquelia dentate* Bedd.—a rare plant of the Western Ghats of India. Indian J Biotech 14(1):123–126
103. Tundis R, Loizza MR, Bonesi M, Peruzzi L, Efferth T (2019) *Daphne striata* Tratt. and *D. mezereum* L.: a study of anti-proliferative activity towards human cancer cells and antioxidant properties. Nat Prod Res 33(12):1809–1812
104. Vallianou NJ, Evangelopoulos A, Schizas N, Kazazis C (2015) Potential anticancer properties and mechanisms of action of curcumin. Anticancer Res 35:645–652
105. Vandermies M, Fickers P (2019) Bioreactor-scale strategies for the production of recombinant protein in the yeast *Yarrowia lipolytica*. Microorganisms 7(2):40
106. Veljkovic B, Dordevic N, Dolicanin Z, Licina B, Topuzovic M, Stankovic M et al (2019) Antioxidant and anticancer properties of leaf and fruit

extracts of the wild raspberry (*Rubus idaeus* L.). Not Bot Hortic Agrobot 47(2):359–367
107. Wang L, Wang J, Fang L, Zang Z, Zhi D, Wang S et al (2014) Anticancer activities of citrus peel polymethoxyflavones related to angiogenesis and others. J Biomed Biotechnol 453972:1–10
108. Weathers PJ, Towler MJ, Xu J (2010) Bench to batch: advances in plant cell culture for producing useful products. App Microbiol Biotechnol 85(5):1339–1351
109. Wetterauer B, Wildi E, Wink M (2018) Production of the anticancer compound camptothecin in root and hairy root cultures of Ophiorrhiza mungos L. In: Kumar N (ed) Biotechnological approaches for medicinal and aromatic plants. Springer, Singapore, pp 303–341
110. Yang XZ, Zheng H, Ye Q, Li RY, Chen Y (2015) Essential oil of Cinnamon exerts anti-cancer activity against head and neck squamous cell carcinoma *via* attenuating epidermal growth factor receptor-tyrosine kinase. JBUON 20(6):1518–1525
111. Yao H, Liu J, Xu S, Zhu Z, Xu J (2017) The structural modification of natural products for novel drug discovery. Expert Opin Drug Discov 12:121–140
112. Yuan H, Ma Q, Ye L, Piao G (2016) The traditional medicine and modern medicine from natural products. Molecules 21(5):E559. https://doi.org/10.3390/molecules21050559
113. Zhong C, Hu D, Hou LB, Song LY, Zhang YJ, Xie Y, Tian L, W. (2017) Phenolic compounds from the rhizomes of *Smilax china* L. and their anti-Inflammatory activity. Molecues 22(515):1–8
114. Zhu J, Wang M, Wen W, Yu R (2015) Biosynthesis and regulation of terpenoid indole alkaloids in *Catharanthus roseus*. Pharmacogn Rev 9(17):24
115. Zuhrotun NF, Astufi M, Murdiati A, Mubarika HS (2017) Anti-proliferation and apoptosis induction of aqueous leaf extract of *Carica papaya* L. on human breast cancer cells MCF-7. Pak J BiSci 20(1):36–41. https://doi.org/10.3923/pjbs.2017.36.41

Publisher's Note