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Abstract

One of the main streams of traditional medicine is herbal medicine; a wide range of medicinal plants and their individual parts are used for therapy. Though not scientifically validated, this traditional medicine practice is much popular in countries such as India, China and Sri Lanka and in many other countries in South, Southeast and Eastern Asia due mainly to its healing capabilities. More recently, scientists initiated the chemical analyses of these medicinal plants, obtaining invaluable results. The latest addition to such investigations is studies on effects of herbal extracts on different types of stem cells. An extensive summary of such reported studies is presented in this chapter, mainly categorizing these into proliferation stimulatory effects on stem cells and inhibitory effects on cancer stem cells (CSCs), where both properties are beneficial in cell therapy procedures. At present, standardizing the products and limited knowledge on the mechanisms of action and pathways of these have critically limited the use of herbal extracts in therapeutics. However, we believe that in the near future scientists would be focusing on herbal remedies to replace the use of synthetic stimulants and cancer drugs to overcome the disadvantages of these, such as toxicity, side effects and exorbitant costs.

Keywords: herbal extracts, stem cell therapy, cellular stimulants, proliferation and differentiation, cancer stem cells

1. Introduction

Traditional medicine is a popular treatment method for a wide range of diseases in many countries due to its claims of therapeutic activity by patients. The knowledge handed over from generation to generation since ancient ages is the foundation of traditional medicine; hence, the methods of treatment vary depending on the country and the region of origin.
In addition, a single region may use different types of traditional medicine due to different ethnic backgrounds of its citizens migrated from different regions of the world.

As the World Health Organization (WHO) defines ‘Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness [1].’

Herbal medicine is one of the main streams of every traditional medicine practice regardless of the different types such as Indian traditional medicine (ITM), Sri Lankan traditional medicine (SLTM), traditional Chinese medicine (TCM), Arabic traditional medicine (ATM), African traditional medicine and South American traditional medicine. According to the WHO, medicinal ingredients of herbal medicine include herbs, herbal materials, herbal preparations and finished herbal products that contain active ingredients as parts of plants or other plant materials or combinations; also, 75% of the world’s population use herbs for their basic healthcare needs [2]. Archaeological proof of history in the use of herbal medicine dates back to more than 5000 years [3], along with evidence from ancient literature such as Arkaprakasa (pharmacology and pharmacy) and Kiemaratatntra (paediatric diseases and management) claimed to be written by the great king Ravana of Sri Lanka where different herbal preparations were introduced for treatment and management of different types of diseases [4]. An in-depth account of the historical events on the use of herbals is reviewed by Petrovska [5]. The same disease could be treated in different countries, with different types of plant-based remedies mainly depending on their indigenous plant varieties and traditional knowledge handed down to generations through thousands of years [6, 7].

Even though history strongly supports the use of herbal medicine, over the last century, traditional knowledge and its effective uses were challenged by Western medical practitioners due to lack of scientific validation of these claims and evidence [2]. However in the recent decades, perspectives on herbal medicine had been evolving into positive thoughts with the isolation of many different effective drugs from plant materials. Existing synthetic drugs are highly expensive, and most of these are required to be replaced due to their instability in vivo [8]. Continuous synthetic drug doses may cause side effects and toxicity [9]; hence, these disadvantages accelerated the search for alternatives derived from natural products. With the technological advances in health and basic sciences, multi screening drug facilities to investigate specific therapeutic activities was made possible. Isolated chemicals and bioactive compounds from plant materials are the main source of modern pharmaceutical drugs, which are either naturally derived or synthetic analogues of existing natural compounds [10]. Among the many different approved drugs derived from herbal material, anticancer drugs [11], antidiabetic drugs [12] and skin care products [13] have maintained topmost status in this long list. In cancer therapy, 25% of the drugs used in the last 20 years are directly derived from plant material [11], and 49% of the antidiabetic drugs approved in the last 10 years were plant derived [12]. Both in developing and developed countries, obesity is becoming a socio-economic burden rendering global populations unhealthy, leading to many non-communicable diseases [14]. There are many weight-reducing supplements prepared by herbal extracts selling in an increased rate in the local markets, even without clinical approval, due to the popularity of the products among the users. Hence, researches are in the timely search of antiobesity herbal preparations [14] as these would flourish as multimillion dollar businesses in the global market.
In order to investigate the different activities of plant-derived extracts, the use of experimental platforms is important prior to clinical trials. Human stem cells are one such experimental platform to investigate therapeutic activities of herbal extracts in vitro. Stem cells with the ability to self-renew and differentiate into many cell lineages have been accepted and extensively used by scientists globally as a reliable tool in their research. Of the many different sources of stem cells, bone marrow stem cells have been used widely in research due to their well-explained characteristics, but the usage paradigm is shifting towards umbilical cord- and cord blood-derived stem cells due to the advantages such as minimum ethical issues, high availability and easy isolation methods of the latter [15]. Since stem cells possess multi-lineage differentiation ability, stimulated differentiation of stem cells could be used to investigate on therapeutics applicable to different types of diseases. For example, human mesenchymal stem cells (hMSCs) could be differentiated into osteocytes, adipocytes and chondrocytes; hence, herbal extracts could be used to investigate the suppression or the stimulation of adipogenic, osteogenic and chondrogenic differentiation properties of stem cells and therefore used to investigate the therapeutic possibilities of diseases related to the above cell lineages in vitro. Human haematopoietic stem cells are the progenitors of cells of blood tissue; hence, those can be differentiated into different blood cell types, and herbal extracts could be used in the above manner to search for therapeutic agents for blood cell-related disorders. Induced pluripotent stem cells (iPSCs), a group of adult somatic cells which are genetically engineered to function as embryonic-like stem cells, are also widely used as disease model stem cell lines in investigations of therapeutic candidates for different disease targets [16]. iPSC-derived cardiomyocytes from patients with cardiovascular diseases and iPSC-derived neurons from patients with neurodegenerative disorders are currently used in high-throughput drug screening [17]. Undifferentiated stem cells are transplanted in order to regenerate tissue in vivo; hence, stimulation factors are important to increase the regeneration speed. The issues of synthetic growth factors and stimulants, i.e. possible side effects, high costs and low availability, remain unchanged; therefore, natural stimulants are preferred. Hence, research is ongoing in search of natural stimulants for stem cells [8]. Furthermore, growth factors, cytokines and vesicles secreted by hMSCs are known as the secretome of hMSCs, and these bioactive factors isolated singly or as a mixture are investigated as potential therapeutic agents, which could reduce the complexities of therapy using cell transplantations [18].

Although it is reported that over 53,000 plant species are used in herbal medicine globally [2], only a few are being tested and reported with scientific proof of their biological activities. The need for merging of traditional herbal medicine knowledge and cutting-edge scientific techniques is essential to produce novel drugs for the benefit of patients. Investigation of mechanisms of actions and pathways, stimulated by herbal extracts, is critical as this would support the scientific validation of such products prior to their market launch. Therefore, this chapter aims to elaborate such research published in the recent decade, in which herbal preparations, extracts and plant-derived bioactive compounds were utilized to produce scientific proof of anti-disease activity, proliferation stimulant activity and differentiation stimulation or suppression of stem cells and their related plausible mechanisms of action. Also, the chapter would identify research gaps related to effects of herbal extracts on stem cells for use in clinical therapy. This chapter harps on the potential of commercializing herbal-based stem cell therapy, which will also be affordable to the developing world.
2. Effects of herbal extracts on human stem cells

2.1. Stimulatory effects of herbal extracts on human stem cells

Our literature search for the use of herbal preparations to stimulate stem cell proliferation and differentiation in clinical trials resulted in no publications or records, explaining that this area of research is at its infancy harping on the vital necessity of this line of research. However, many studies have been reported on the use of animal models with end results of in vitro studies, cross-linking the above-mentioned research areas, suggesting that the impending phases of research would hopefully culminate in clinical trials, leading to natural products being marketed as commercial stem cell-stimulating agents.

There are several reviews published summarizing the effects of different herbal extracts and their isolated bioactive compounds on human and other mammalian stem cells isolated from different sources. Our review published in 2016 elaborates on osteogenic, anti-adipogenic, neurogenic, endothelial/vascular genesis, angiogenesis and proliferative effects of herbal extracts on human mesenchymal stem cells mostly confirmed by RNA expression studies [8]. Dried root of Korean herb Dipsacus asper had been used in Korean traditional medicine for the treatment of bone fracture and the crude extract, and an isolated compound from the herb hedraganin-3-O-(2-O-acetyl)-α-L-arabinopyranoside demonstrated the osteogenic differentiation ability on bone marrow-derived hMSCs via the upregulation of bone-specific proteins and alkaline phosphatase activity [19]. Aloe emodin, present in Aloe latex, showed anti-adipogenic activity on hMSCs by reducing expression levels of mRNAs (resistin, adiponectin, aP(2), lipoprotein lipase, PPARγ and tumour necrosis factor-α) involved in adipogenic pathways [20]. Treatment of adipose-derived hMSCs with dried root extract of Angelica sinensis, an herb used in traditional Chinese medicine, resulted in significantly higher differentiation of neural-like cells than a commonly used neural inducer, butylated hydroxyanisole [21]. The neuroprotective ability of the same extract was proven by decreased induced neurotoxicity in cultured cortical neurons, increasing the extract’s value as a potential candidate in treating neurodegenerative disorders [22]. A patent was obtained for endothelial differentiation of hMSCs treated with olive leaf extract with overexpression of gene vascular endothelial growth factor, PCAM, platelet-derived growth factor receptor and vascular endothelial growth factor receptor (VEGFR)-1 [23]. An updated list of herbals and mechanism of actions on MSCs, as well as a list of phytochemicals (resveratrol, genistein, naringin, icariin) isolated from plant extracts, were presented in a similar review published in 2017 [24]. As elaborated here, all four isolated compounds had proven their ability to differentiate MSCs into osteoblasts and osteocytes, possibly through the Wnt signalling pathway, upregulating gene expression of RUNX2 and Sirt-1 genes [25–27]. Combined therapy of adipose-derived hMSCs with icariin showed significantly improved survival rates of hMSCs as well as increased expression of endothelial markers and smooth muscle markers in rat models with diabetes mellitus-induced erectile dysfunction (DMED) inhibiting oxidative stress via the regulation of PI3K/Akt-STAT3 signal pathway [28]. A previous review published in 2014 demonstrated the well-established link between herbal preparations used in Ayurveda for a wide array of disorders with their proliferation and differentiation effects which were utilized in similar capacities on stem cell differentiation and proliferation, providing scientific proof of thousands of years old Ayurvedic predictions and practices [29]. Rasayana, the branch of Ayurveda
which explains rejuvenation and immunomodulation, has listed the use of approximately 200 herbs [28] which could be investigated for their regeneration capacities on stem cells. *Medhya Rasayana*, an intellectual/retention rejuvenation therapy method in Ayurveda that consists of four herbal plants, could be used individually or in combination [30]. Studies on stem cells treated with *Medhya Rasayana* extracts have shown the expression of nestin on stem cells, an early neural stem cell marker [29], confirming the ability of *Medhya* herbs to treat disorders related to the neural system by increasing the differentiation ability of stem cells.

A growing concern of ameliorating radiation-induced normal tissue injury is arising as it affects the well-being of cancer patients. Stem cell therapy is used to replace these cells and tissues, and many examples are elaborated in the review of Benderitter *et al.* [31]. Authors have reviewed a number of studies related to ameliorating radiation-induced myelopathy by transplanting neural stem cells to the spinal code [32], potential applications of transplanting salivary gland stem cells in patients with radiation-induced xerostomia [31], potential benefits of transplanting stem cells and biomaterial in animal models with osteoradionecrosis [33] and transplanting autologous fat drafts including adipose-derived stem cells to treat radiation-induced late skin complications [34]. As herbal extracts had proven their differentiation aiding capabilities in *in vitro* studies, they could act as stimulants to produce increased numbers of stem cells required for patient transplantations. The following figure illustrates the different sources of human mesenchymal stem cells (hMSCs) and their differentiation capabilities with advantages and disadvantages of herbal stimulants and synthetic stimulants (Figure 1) [8].

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**Figure 1.** A glimpse of hMSC sources and their differentiation capabilities stimulated with herbal extracts or synthetic stimulants (Courtesy: Udalamaththa *et al.* [8]).
Although most of the reported research was on hMSCs, haematopoietic stem cells (HSCs) are also being investigated for their properties of proliferation and differentiation when treated with herbal extracts and their isolated compounds. Proliferation, differentiation and in vitro expansion of healthy hHSCs are important as many haematological malignancies disrupt the healthy hHSC populations. A review that summarizes a wide range of research publications on the use of Chinese herbal medicine (CHM) to promote recovery after HSC transplantation had elaborated the positive results of herbal extracts from plants such as Sheng Di Huang (*Rehmannia glutinosa*), Bai Zhu (*Atractylodes macrocephala*), Ren Shen (*Panax*), Dang Shen (*Codonopsis pilosula*), Mai Men Dong (*Ophiopogon japonicus*), Dang Gui (*Angelica sinensis*), Tai Zi Shen (*Pseudostellaria heterophylla*), Huang Qi (*Astragalus membranaceus*) and Ejiao (*Equus asinus*) [35]. A study on autologous and allogenic HSC transplanted in patients with chronic granulocytic leukaemia, acute non-lymphocytic leukaemia and lymphoma were treated with CHM concluded that treating with CHM reduces complications of transplantations and promotes recovery of haematopoietic functions [36]. More research on various other HSC transplantations against haematological malignancies such as severe aplastic anaemia patients [37], patients with myelodysplastic syndrome [38] and acute paediatric leukaemia [39] were cited herein [35], which had given positive results on patient survival rates, reduction of complications and increasing functional properties of haematopoietic cells. However, most of these studies were based on a low number of samples; hence, the need to perform such studies in large populations arises in order to validate and standardize the CHM procedures. In vitro studies and animal model studies had also been reported on HSC proliferation and differentiation to gather more scientific evidence to support small local clinical trials performed in isolation in individual countries. EMSA eritin, a polyherbal formulation had increased proliferation of HSC in irradiated BALB/c mice in vivo and triggered differentiation into the lymphopoiesis lineages [40]. Inducing of proliferation and attenuating of apoptosis were observed when an immune-mediated aplastic anaemia mouse model was treated with a modified Chinese herbal formula prepared with Radix astragali, Radix *Angelicae sinensis* and *Coptis chinensis* Franch [41].

Although stem cell therapy had boosted disease therapy into the next level of modern therapeutic medicine, a major limitation is their poor survival after transplantation into the host, which could be resolved by supplementing the microenvironment with vitamins and other antioxidants [29] and other preconditioning strategies such as exposure to hypoxic conditions, oxidative stress and heat shock treatments [42]. Scientists are studying natural plant extracts and their isolated compounds as alternatives to synthetic growth factors and other stimulants to precondition the microenvironment for the survival of stem cells in vivo, as there are many reports on the presence of a wide array of beneficial phytochemicals in plants. Pretreatment of adipose-derived hMSCs with *C. setidens* herbal extract had resulted in increased survival of hMSCs by inhibiting ROS-induced apoptosis, suggesting the suitability of the extract to prevent ROS-induced oxidative stress by regulating the oxidative stress-associated signalling pathway and suppressing the apoptosis-associated signal pathway [43]. Extract of *Origanum vulgare* had protected murine mesenchymal stem cells from oxidative stress when precon-
ditioned with high doses via significantly decreasing caspase-3 activity [44]. *Tinospora cordifolia* and *Withania somnifera*, two widely used herbs used in Ayurveda for rejuvenating and anti-ageing treatment, had shown increase in proliferation and inhibition of senescence in WJ-MSCs *in vitro* [45], suggesting that pretreatment with these herbals would aid in *in vivo* transplantation procedures.

### 2.2. Inhibitory effects of herbal extracts on cancer stem cells

Cancer stem cells (CSCs), the cells which are capable of self-renewal and produce the heterogeneous lineage of cancer cells [46], has become the most complicated issue in cancer therapy. A number of studies were reported which resulted in the reduction of cancer cells with the treatment of isolated phytochemicals such as epigallocatechin-3-gallate (EGCG), curcumin, resveratrol, lycopene, pomegranate extracts, luteolin, genistein, piperin, β-carotene and sulforaphane [45]. Specifically, sulforaphane, a phytochemical isolated from broccoli, had apoptosis-inducing effects on pancreatic CSCs [47] and could target breast CSCs effectively [48].

However, in this scenario, scientists are changing their approach in the search for natural products by trying to select herbal extracts and preparations known to be effective against cancers in traditional medicine. This approach would be advantageous for both ends of traditional medicine and modern therapeutics, as traditional medicine will have a chance of proving the remedies in a scientific platform and also the modern therapeutics would have the benefit of using time tested anticancer remedies rather than screening thousands of plant extracts for this purpose without any clues. A review on targeting CSCs using TCM remedies and their active compounds had elaborated several approaches of herbal remedies acting on CSCs. Reversion of drug resistance of CSCs, inducing cell death and inhibiting cell proliferation, inhibiting metastasis and targeting CSCs-related miRNAs are the explained methods of TCM remedies targeting CSCs [49]. Berberine liposomes, isolated from rhizome of *Coptis chinensis*, showed anticancer effects on human breast CSCs transplanted in nude mice by penetrating the cell membrane, accumulating in mitochondria of CSCs and resulting in reversion of drug resistance and apoptotic pathway inducing cell death and inhibiting cell proliferation [50]. Curcumin and epigallocatechin gallate (EGCG) had synergistically targeted breast CSCs by downregulating stemness genes and inducing differentiation of these into non-stem cells [51]. Prostate cancer metastasis had been reduced by a combination of quercetin, extracted from *Dysosma veitchii* and EGCG by reducing activity of LEF-1/TCF responsive receptor [52]. Honokiol, a lignan isolated from *Magnolia officinalis*, had inhibited renal cancer metastasis by regulating miR-141/ZEB2 signalling [53]. Triphala, a widely used formulation in Ayurveda, had shown anticancer properties on human colon cancer stem cells by p53-independent proliferation inhibition and apoptosis inducing [54]. Also, a Sri Lankan group of scientists had investigated on anticancer properties of gedunin, a major compound found in *Azadirachta indica*, which confirmed its apoptotic-inducing properties against human embryonal carcinoma cells—a cancer stem cell model [55].
2.3. Commercial herbal products with claims of stem cell rejuvenation

Many herbal products are commercialized with claims to be rejuvenating adult stem cells which are considered as stem cell supplements. The first stem cell enhancer was developed and patented by Dr. Sahelian of Stemtech HealthSciences, Inc. in 2005 [56] which included extracts of freshwater microalgae and marine macroalgae [57]. Stem Cell 100® is a patent pending product prepared from bioactive compounds of herbal plants Astragalus membranaceus, Vaccinium, Pine bark, Camellia sinensis, Pterocarpus marsupium, Polygonum multiflorum, Schisandra, Fo-Ti root and Drynaria rhizome mainly derived from TCM [58]. ProxyStem is another patent pending nutraceutical stem cell supplement with claims to be working on pro-inflammatory pathways, endothelial cell health, oxidative stress protection, mitochondrial function and artery support [59].

Another product, NutraStem Active, was awarded a patent for claims of its ability to promote adult stem cells with its four ingredients—blueberry extract, green tea extract, L-carnosine and vitamin D3 [60]. Stem-Kine, a clinically proven stem cell supplement, includes ellagic acid which protects stem cells from free radicals [61]; it is a polyphenol compound extracted from mainly a plant of the berry family [62].

3. Pros and cons of using herbal remedies to stimulate stem cells

Traditional herbal treatment provides a straightforward method to identify the link between plant/herbal remedies and their use in curing different diseases. Modern scientists now use the same strategy to identify herbal plants and their isolated compounds which could be used as stem cell stimulants for much needed stem cell therapeutic procedures. Studies were initiated in this line of research in developed countries as well as in the developing countries acquiring their own traditional herbal treatment knowledge. China seems to be much ahead in this hybrid system of research using Chinese traditional herbs/isolated compounds and cutting-edge screening technologies. Although there is a plethora of internationally published research by research groups from China, many clinical trials and small population studies seem to be concealed from the rest of the world as these reports are published in local journals in their native language [35]. China is not alone in this exercise. Other countries such as Iran and Pakistan too with rich traditional medicine cultures and also into stem cell research are posing the same issue, as the data they produce are not communicated to the international scientific community. This is an unfortunate situation which could be rectified to be more productive through collaborative research with the rest of the world.

In certain instances, developing countries offer their knowledge of traditional herbal medicine together with their rich local plant diversity to collaborate with developed countries to obtain cutting-edge technologies to achieve high potential results in their research. However, the strict local regulations and policies on shipping indigenous plant material or their compounds in developing countries, in order to protect their own plant species, had restricted this productive collaborative research framework, as this process is lengthy which would lead to late initiation of laboratory investigations.
Another concern is that of the withdrawal of traditional herbal practitioners from providing information on their herbal remedies to the scientists for investigations; it is the latter who have the ability to scientifically prove that these remedies are actually therapeutically potent. Traditional practices are said to be handed down from generation to generation within families, and most of these practitioners treat patients *pro bono*, as a social service. Since these practitioners claim to have satisfactory results from providing such treatment, they have no reason to give away their herbal remedies, which had been a family secret for over hundreds of years. However, the modern graduates of traditional medicine are more into scientifically validating their treatment methods, as it is beneficial for their practice to have scientifically proven results to compete with Western medicine practitioners. Most traditional medicine practitioners vary the constituents of herbal preparations and the ratios used in their prescriptions even for the same disease depending on the patient’s individual constitution, indicative of the practise of ‘personalized/precision medicine’. ‘Ayugenomics’ irrevocably established that a genetic basis did indeed exist to the said individual constitutions [63]; differential DNA methylation signatures in the three distinct ‘prakriti’ phenotypes (based on distinctly descriptive physiological, psychological and anatomical features of different individuals) demonstrated the epigenetic basis of traditional human classification in Ayurveda with relevance to personalized medicine [64]. Yet, allopathic medicine strongly believes in standard preparations where only the dose is varied among individual patients. Hence, there arises the question whether modern standardized herbal preparations would be universally effective on every patient.

Nevertheless, herbal remedies that were scientifically investigated for their properties with elucidated mechanisms and pathways of action too may face further obstacles prior to their market launch. As mentioned in the review of Udalamaththa *et al.*, a large-scale manufacturing process may reduce the crude properties of herbal remedies, solvents used to prepare extracts may produce adverse effects when used in therapy, complexity and variability of bioactive compounds may make clinical applications challenging [8]. As standardization of herbal products is a must prior to the market launch, similar and stringent regulations will be applied to herbal stem cell stimulants which are to be used in therapy.

Yet, despite all issues involved, pharmaceutical companies are competing for patents and commercializing herbal stimulants, supplements and many more drugs which could be used in stem cell therapy.

4. Conclusion

Herbal medicine has at all times been a trusted treatment method from ancient eras. The paucity of the use of herbal medicine or related treatment methods in allopathic medicine practices or other types of therapy using cutting-edge technology may pose the ‘missing part of the puzzle’ which scientists and clinicians have strived to solve. However, in recent years, both traditional medicine and novel technologies in synergy have resulted in beneficial outcomes advantageous to the patients. Examples presented in this chapter provide a glimpse of recent studies where herbal medicine and stem cells have been amalgamated in search of treatment against ‘incurable diseases’. Although the use of medicinal plants in stem cell
research is in its infancy, with small population studies within local communities, with low numbers of related patents and many complexities in application in a clinical setting, the attraction of this area of research has never ebbed due to the promising results emerging from basic scientific research. Preliminary trials leading to the initiation of in-depth studies may well result in inexpensive, available, nontoxic drugs, stimulants and supplements useful in stem cell therapy.

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Author details

Preethi Vidya Udagama* and Vindya Udalamaththa

*Address all correspondence to: preethi@zoology.cmb.ac.lk

Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, Colombo, Sri Lanka

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