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Review

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Posted Date: 26 January 2023

doi: 10.20944/preprints202301.0462.v1

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Review

Plant-Based Bone Grafting: The Future of Bone Healing

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Abstract: Objectives Bone fractures are very common diseases, which can be caused by impact injuries or physiological disorders. Thus, the present review aimed to study the use of medicinal plants in the healing mechanism of bone fractures. **Evidence acquisition** Through research in the PubMed, Google Academic, and Scielo databases, this article reviews 11 ethnopharmacological studies and 34 preclinical studies on the biological actions of different plants in bone fracture healing mechanism. **Results** Indian tribes have highlighted in the plants ethnopharmacological study for various diseases, including bone fractures. However, despite the large citations of traditional use, technical-scientific studies are still scarce in the literature. Chenopodium ambrosioides, Piper sarmentosum, quadrangular Cissus, Ricinus communis and Radix salvia miltiorrhiza plants were the most studied in the literature regarding their osteogenic, angiogenic, anti-inflammatory and remodeling effects, acting on bone receptors, stimulating bone metabolism, increasing minerals uptake, and assisting in free radicals breakdown. **Conclusion** Thus, the medicinal plants use is promising in the field of bone regeneration, as well as being alternative when conventional therapies are unfeasible, increasing herbal medicines demand and popularity.

Keywords: bone healing; bone fracture; herbal medicine; osteogenesis; plants; rats

Introduction

Bone fracture refers to the separation of a bone into two or more segments. It can be caused by high impact stress, such as traumatic injury due to various accidents that can affect man or result from certain pathological conditions that weaken bones, such as osteoporosis that cause fractures due to bone fragility [1] . Musculoskeletal injuries represent US$ 56 billion each year, of which US$ 21 billion goes to fracture treatment, second only to heart disease [2]. Although advances in bone injuries management have improved survival rates, subsequent complications remain challenging, requiring further research with alternative treatments through new molecules that act on bone repair [3].

Healing of bone defects can be improved with plants that control inflammation, promote angiogenesis, and stimulate bone regeneration. [4,5] Many of these plants, traditionally used by indigenous and underprivileged communities, have been studied and validated by the scientific community as raw material sources and new biomaterial prototypes generation [4,6,7].

The traditional forest knowledge system has been poorly studied, except as clues to asset discovery, although it has been the basis of human livelihoods and medicines for centuries. Chemical and pharmacological investigations of these traditional medicines have often provided new bioactive compounds for modern therapies. Prostrain, an herbal treatment against HIV that activates the pool

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of latently infected T cells, was discovered from ethnobotanical work in Samoa[8,9], which makes these means of study important in the new drugs discovery.

The fracture treatment aims to achieve rapid bone union [10] and, for this purpose, various and sophisticated methods have been developed, such as the use of mechanical stimulation, electrical and electromagnetic devices, low intensity ultrasound, bone morphogenetic protein implantation and grafts, autogenous, allogeneic, synthetic, and among other developed therapies [11]. Although they positively promote bone healing, they have limited clinical uses because of their high costs, difficult-to-handle instrumentation, high degree of morbidity and restrictions, and uniform results lack [12,13].

This review aimed to address the data published in medicinal plants specialized literature and their role in bone healing. Thus, it included studies available in the MEDLINE - PubMed (U. S. National Library of Medicine, National Institutes of Health), Google Scholar and Scielo databases. The descriptors used were "ethnopharmacology and bone fractures", "medicinal plants and bone regeneration", "medicinal plants and bone healing", "herbal medicine and bone healing" and "plants and bone grafts". Studies published between the years 2006 to 2022 were included. The combination of the above descriptors allowed to select studies related to bone healing and medicinal plants. Inclusion criteria applied in this study were ethnopharmacological studies, preclinical trials, primary and interventional studies with medicinal plants. Exclusion criteria were unclear data sources, duplicate data, theoretical discussion, case report, abstracts, conferences, and letters to editors. In addition, other relevant work on bone regeneration has been included to introduce the subject.

Bone healing

Skeletal tissue has a great regenerative capacity that occurs through a cascade of biological and molecular events in coordinated and complex form, being one of the few tissues that can heal without forming fibrous scar tissue, regaining the mechanical properties of bone again [14]. There are two ways to normalize bone injury, intramembranous healing or direct or more commonly indirect or secondary. Intramembranous healing (direct) occurs with minimal callus formation and movement of the fracture edges and, therefore, requires direct contact of cells in the cortex. However, most fractures heal secondary or indirectly, since secondary healing has presence of interfragmentary movement, creating relative stability and greater periosteal reaction with greater presence of bone callus [14] which is, eventually, remodeled to mature bone. [15]

Fracture healing initially involves an anabolic phase, characterized by an increase in newly formed bone callus tissue, that is related to stem cells recruitment and differentiation that form tissues from skeletal and vascular cells [3,16], occurring gradually and overlapping, involving the stages of acute inflammation, revascularization and calcification, ossification and remodeling, and bone structure formation [17]. After the fracture, the surrounding bone and soft tissue vascular injury causes hypoperfusion in the adjacent areas, activating the coagulation cascade and leading to the formation of a platelet- and macrophage-rich hematoma. Macrophage-derived cytokines initiate an inflammatory response, including increased blood flow and vascular permeability at the fracture site, where mechanical and molecular stimuli will drive future events [18].

Molecular events, characterized by the expression of numerous cytokines and proinflammatory factors, coordinate these complex pathways, where tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-11 and IL-18 are responsible for the initial inflammatory response [11]. Mesenchymal stem cells are recruited through the surrounding soft tissues, later differing in osteogenic and cartilaginous cells involved in the periosteal bone callus generation [19].

Revascularization is an essential component of bone healing, which occurs through molecular pathways that require angiopoietin or vascular endothelial growth factor (VEGF) [20]. However, VEGF has been shown to be of paramount importance in the process of bone repair in studies involving animal models [21,22].

After the anabolic phase, catabolic activities predominate and are characterized by the tissue volume reduction of the newly formed bone callus. During the predominantly catabolic phase, there is cartilage resorption, despite specific anabolic processes still occur with secondary bone formation and continuous primary angiogenesis as nascent bone tissues replace cartilage [23].

When bone remodeling begins, the mineralized matrix produced during primary bone formation is resorbed by osteoclasts and, then, the bone deposited during the cartilage resorption period is also resorbed. As bone tissue continues to reabsorb, this prolonged period is characterized by osteoblastic and osteoclastic activity cycles, in which callus tissues are remodeled into the original cortex of the bone structure, called "coupled remodeling" [24]. During this time, the medullary space is reestablished, occurs original hematopoietic tissue structuration and extensive bone vascular remodeling. The vascular bed enlargement regresses, and the high vascular flow returns to the pre-injury level. Although these processes occur consecutively, they overlap substantially [25].

Ethnopharmacological use of medicinal plants in osteointegration

About 50,000 plant species have been registered for their medicinal uses and the World Health Organization estimates that about 80% of the world's population still depends on such plants as their primary source of medicines [26].

The ecosystem services provided by forests are vital for survival and human well-being, being also indispensable for the conservation of biodiversity. Indigenous peoples, underprivileged regions, where it is difficult to obtain traditional medicines, and worldwide use the forest as different sources, such as agriculture, fishing, hunting, medicines, building materials and implements [27].

Medicinal plants comprise more than 25% of medicines on the market. In the last decade, there has been an increase in the international herbal medicine market, in line with pharmaceutical and clinical research, although it varies between countries [27]. Thus, the community interaction that makes traditional use of these plant species and researchers is relevant in order to correlate and use all scientific developments and ethnopharmacological concepts for new therapies generation [5]

Ethnopharmacological studies conducted in communities and tribes worldwide, such as India, Cameroon, Philipinas, Bangladesh, Brazil and Southeast Asia, were made by researchers. They evaluated the traditional medicinal plants use in the treatment of various diseases, focusing on plants with bone fracture healing activity, used in different ways, such as local application in the form of pastes and systemic use by infusions and teas, according to the bone fracture nature. Various species and different parts of plants used by these populations were reported [Table 1].

Table 1. Ethnopharmacological studies of medicinal plants used to heal bone fractures.

Scientific Name	Family	Plant part	Local	Country	References
Merremia umbellate (L.)	Convolvulaceae	Leaves	Tripura	India	[97]
Microcos paniculata (L.)	Tilliaceae	Leaves	Tripura	India	[97]
Cissus quadrangulares (Linn.)	Vitaceae	Tendrils	Dhemaji	India	[27]
Alangium salvifolium (L. f.)	Alangiaceae	Bark / Root	Nalgonda e Warangal	India	[98]
Acampe praemorsa (Roxb)	Orchidaceae	*	Visakhapatnam	India	[99]
Desmodium triflorum (L.)	Moraceae	Plant	Visakhapatnam	India	[99]
Ficus benghalensis (L.)	Fabaceae		Munta mandu	India	[99]
Tridax procumbens (L.)	Asteraceae	Leaves	Gaddichamanthi	India	[99]
Pholidota articulata (Lindl.)	Orchidaceae	Leaves	Kumaon	India	[100]
Coelogyne cristata (Lindl.)	Orchidaceae	Roots	Kumaon	India	[100]
Vanda cristata (Lindl.)	Orchidaceae	Leaves	Kumaon	India	[100]

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Ulmus wallichiana (Planch.)	Ulmaceae	Stem bark	Kumaon	India	[100]
Blepharis integrifolia (L.f.)	Acanthaceae	Whole plant	Western Ghats	India	[101]
Dicliptera paniculata (Forssk.)	Acanthaceae	Leaf	Western Ghats	India	[101]
Holigarna grahamii (Wight)	Anacardiaceae	Bark	Western Ghats	India	[101]
Amorphophallus paeoniifolius (Dennst.)	Araceae	Grain	Western Ghats	India	[101]
Pothos scandens (L.)	Araceae	Whole plant	Western Ghats	India	[101]
Terminalia cuneata (Roxb.)	Combretaceae	Stem bark	Western Ghats	India	[101]
Diospyros montana (Roxb.)	Ebenaceae	Leaf	Western Ghats	India	[101]
Antidesma acidum (Retz.)	Euphorbiaceae	Bark	Western Ghats	India	[101]
Bridelia stipularis (L.)	Euphorbiaceae	Bark	Western Ghats	India	[101]
Baliospermum solanifolium (Willd.)	Euphorbiaceae	Root	Western Ghats	India	[101]
Glochidion heyneanum (Wight & Arnott)	Euphorbiaceae	Stem	Western Ghats	India	[101]
Abrus precatorious (L.)	Fabaceae	Roots and seeds	Western Ghats	India	[101]
Cassia fistula (L.)	Fabaceae	Stem bark	Western Ghats	India	[101]
Senna tora (L.)	Fabaceae	Leaf	Western Ghats	India	[101]
Millettia pinnata (L.)	Fabaceae	Leaf	Western Ghats	India	[101]
Tamarindus indica (L.)	Fabaceae	Leaf	Western Ghats	India	[101]
Casearia tomentosa (Roxb.)	Flacourtiaceae	Leaf	Western Ghats	India	[101]
Ocimum basilicum (L.)	Lamiaceae	Leaf	Western Ghats	India	[101]
Cinnamomum wightii (Meisn.)	Lauraceae	Stalk	Western Ghats	India	[101]
Persea macranta (Meisn.)	Lauraceae	Stem bark	Western Ghats	India	[101]
Tinospora cordifolia (Willd.)	Menispermaceae	Stem	Western Ghats	India	[101]
Tinospora sinensis (Lour.)	Menispermaceae	Stem	Western Ghats	India	[101]
Ficus benghalensis (L.)	Moraceae	Leaf	Western Ghats	India	[101]
Moringa oleífera (Lam.)	Moringaceae	Gum and root	Western Ghats	India	[101]
Nyctanthes arbor-tristis (L.)	Oleaceae	Root	Western Ghats	India	[101]
Cynodon dactylon (L.)	Poaceae	Whole plant	Western Ghats	India	[101]
Citrus limon (L.)	Rutaceae	Fruit	Western Ghats	India	[101]
Zanthoxylum rhetsa (Roxb.)	Rutaceae	Seeds	Western Ghats	India	[101]
Cissus quadrangularis (L.)	Vitaceae	Stem	Western Ghats	India	[101]
Urena lobata (L.)	Malvaceae	Whole plant	West Cameroon	Cameroon	[6]

Elephantopus mollis (Kunth.)	Asteraceae	Leaves or branch	West Cameroon	Cameroon	[6]
Momordica multiflora (Hook.f)	Cucurbitaceae	Whole plant	West Cameroon	Cameroon	[6]
Asystasia gangetica (L.)	Acanthaceae	Whole plant	West Cameroon	Cameroon	[6]
Brillantaisia ovariensis (P. Beauv.)	Acanthaceae	Whole plant	West Cameroon	Cameroon	[6]
Spilanthes africana (DC.)	Asteraceae	Whole plant	West Cameroon	Cameroon	[6]
Lasia aculeata (Lour.)	Araceae	Root	*	Bangladesh	[102]
Pothos scandens (L.)	Araceae	Leaf and bark	*	Bangladesh	
Chenopodium ambrosioides (L.)	Chenopodiaceae	Leaves	Piauí	Brazil	[103]
Chloranthus glaber (Thunb.)	Chloranthaceae	*	*	Philippines	[104]
Cryptolepis buchanani (Roem. & Schult.)	Asclepiadaceae	Whole plant	Arunachal Pradesh	Southeast Asia	[5]

* Not detected.

Medicinal plants the bone repair process

Although the traditional use of herbal extracts for the bone fracture repair has abundant mention of their positive effects, their validation through controlled studies is still scarce. Thus, medicinal plants use as alternative therapy is promising in the field of bone regeneration, since they present biocompatibility, ease of application and storage, and have been shown to favor osteogenesis [28].

Plants used in the fractures treatment must have important characteristics through their active constituents, presenting the following activities: antimicrobial; antioxidant, important in free radical scavenging that contribute to bone healing delay; osteogenic activity, contributing to increased osteoblast proliferation, osteocytes and osteoclasts; angiogenic activity, acting on the supply of nutrients to the fracture bed, stimulating collagen production; estrogenic activity, important in cases of fractures caused by metabolic diseases as osteoporosis [29–31]; anti-inflammatory, through edema control and pain relief by the chemical mediators production, such as arachidonic acid metabolites and cytokines [32].

The medicinal plants reported in the researched literature and their main activities and mechanisms in the bone repair process have been listed [Table 2].

Dysphania ambrosioides

Dysphania ambrosioides (L.) Mosyakin and Clemants (syn. *Chenopodium ambrosioides* L.) is a widely reported plant for its traditional use by the population in Brazil and Latin America [33] for the treatment of inflammatory conditions [34] and/or bruises and fractures [35,36]. In addition, *D. ambrosioides* is ranked 17th on the National Register of National Health System Interest Plants (RENISUS), a compiled by the Brazilian government consisting of a list of 71 plant species used in folk medicine for health conditions alternative treatment [37].

Experimental studies have shown that *D. ambrosioides* exerts healing activities on bone tissue. In a study reported by Pinheiro Neto et al., [38] where osteointegration was studied through technical analysis, it was possible to observe the highest bone callus growth under X-ray within 30 days, and better results in biomechanical analysis with greater tensile strength of bone in the *D. ambrosioides* graft group, when compared to other bone grafts already used in surgical routine such as *Ricinus communis* polyurethane (castor oil) and autogenous bone marrow.

Experimental results also revealed higher activity of alkaline phosphatase and osteocalcin during early fracture healing, as well as early osteogenesis and increased osteoblastic activity at fracture focus, accelerating the healing process. Histologically proven when the immature bone was observed in *D. ambrosioides* graft within 30 days, while cartilaginous tissue formation predominated

doi:10.20944/preprints202301.0462.v1

longer in other groups, and the largest amount of type I collagen was in the *D. ambrosioides* graft during treatment. Strong presence of flavonoids and appreciable antioxidant activity were detected. These results highlight *D. ambrosioides* as an important osteointegrating agent, demonstrating the importance of medicinal plants in the phytomedicines production [28]. This plant is rich in phenolic compounds, such as flavonoids and anthocyanidins, as well as monoterpenes, among others. Thus, their presence in the *D. ambrosioides* graft may be involved in the bone neoformation process [39].

Several plant extracts contain anthocyanidins, flavonoids and phenolic acids that act in the initial healing process by moderating superoxide anions and, later, increasing vascular endothelial growth factor (VEGF) expression and blood flow, promoting the repair process. They have also been shown to be able to bind to the estrogen receptor (ER), neutralizing the deleterious effects of estrogen deficiency on bone, and are promising molecules for use in bone fracture therapies [40]. In addition, this metabolites has had direct effects on bone metabolism, such as decreased bone resorption by osteoclasts, promotion of differentiation and mineralization of pro-osteoblastic cells, and an increase in alkaline phosphatase activity [41].

Piper sarmentosum

Piper sarmentosum (Ps) is a widely distributed plant in Southeast Asia and is generally used as a flavoring agent in food [42]. In Malaysia, its extract has been used to treat toothache, cough and menstrual disorders. [43] Extracts from different parts of the Ps plant have been reported to have antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties [44]. Ps methanolic extract is rich in phenolic compounds, such as naringenin, also considered a phytoestrogen [45], belonging to the flavonoids group, which exhibit high free radical scavenging activity [46].

The histological analysis of ovariectomized rats that received the Ps extract studied by Estai et al. [47] indicated greater callus maturity and restoration of pre-fracture properties in an estrogen deficient state. Ps treatment reduces the ROS level at the fracture site, which can prevent oxidative stress in healing process of osteoporotic femoral fracture. In addition, ovariectomized rats treated with Ps showed mature bone tissue with few cartilaginous cells in some parts of the fracture callus, with immature bone remodeling to lamellar bone, suggesting that, through the Ps antioxidant action and flavonoids content, lipid peroxidation in fracture callus was prevented, reducing ROS level. Thus, free radical scavenging activities of *P. sarmentosum* flavonoids can play an important role in ROS reduction and oxidative stress prevention during fracture healing.

Estrogen deficiency acts on oxidative stress, increasing the level of reactive oxygen species (ROS) and hydrogen peroxide (H2O2), inducing osteoclast activity with greater bone resorption, delaying fracture healing [48], culminating in nonunion, malunion or delayed union of fractures. Therefore, flavonoid-rich diets, such as rutin, have been shown to prevent the induction of bone loss in ovariectomized rats [49], as isoflavones prevent menopause-induced bone loss in women, an effect attributed to their antioxidant activity [50].

Cissus quadrangularis

Cissus quadrangularis Linn is a perennial plant found mainly in hottest parts of the world such as India, Sri Lanka, Tropical Africa, South Africa, Thailand, Java and Philippines [51]. The plant is mentioned in ancient medicine systems, such as Ayurveda, and is useful for treating bloody diarrhea, skin problems, earaches, hemorrhoids, irregular menstruation and accelerates bone fractures healing. Plant phytoconstituents are notable and support their various therapeutic activities, beyond bone remineralization [52].

C. quadrangularis phytochemical screening revealed the presence of iridoids, stilbene derivatives, ketoesterols, triterpenes, alkaloids, fatty acids, methyl esters, flavonoids, and other compounds [53]. Ketosteroids and triterpenoids also are considered phytoestrogens and are important molecules in bone healing, due to their role in bone estrogen receptors. They have been shown to accelerate ossification and remodeling, stimulating metabolism and increasing the uptake of calcium, phosphorus and strontium minerals by osteoblasts [54,55].

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The main traditional use of *C. quadrangularis* is in bone remineralization and fracture. Therefore, there are several studies that have already established the ancient use of this plant in this interest area. A study of petroleum ether extract revealed the effects of *C. quadrangularis* on bone cell proliferation and mineralization. The experiment was performed by isolating bone marrow mesenchymal stem cells (MSC) from male Wistar rats, and MSC groups could differentiate with and without addition of *Cissus quadrangularis* petroleum ether extract. The study concluded that groups treated with *C. quadrangularis* extract had a much higher proliferation rate and subsequent differentiation into osteoblasts, which was identified using alkaline phosphatase (ALP), an indicator for osteoblast activity, as a biomarker. MSC differentiation for osteoblasts was absent in groups not treated with plant petroleum ether extract, revealing that treated groups with petroleum ether extract have a very high rate of mineralization [56].

Beneficial effects of *C. quadrangularis* plant extract were found in a clinical study with patients who had mandibular fractures compared to the placebo group. The acceleration in fracture healing was attributed to stimulation of all cells of mesenchymal origin, as fibroblasts, chondroblasts and osteoblasts. Steroid anabolic principle of *C. quandrangularis* showed a marked influence on fracture healing rate, influencing the early regeneration of all connective tissues involved in healing and faster callus mineralization. There was a shortening of about two weeks in bone healing duration [57].

Another study with patients with mandibular fractures, Singh et al. [58] evaluated fracture healing by osteopontin expression during treatment with *C. quandrangularis* extract capsules by oral via, when compared with placebo group. Systemic use of *C. quandrangularis* in rats caused complete restoration of normal bone composition after fracture within 4 weeks, while controls required 6 weeks. There was a shortening of about 2 weeks in bone healing duration. Total weight of fractured bone also decreased to normal much earlier than controls, indicating faster bone remodeling. All events, such as fibroblastic, collagenous and osteochondral, were accelerated in about 10-14 days in the treated group.

Cannabis sativa

Cannabis sativa L. (Cs) has been used for thousands of years for medicinal and recreational purposes. Reports of its medicinal use range from Ancient China, Medieval Persia and 14th-century Europe, and include its use to treat headaches, fever, gastrointestinal problems, malaria and even as an antibiotic. The plant is made up of over 100 compounds, but the main ones are 11-Tetrahydrocannabinol (THC) and Cannabidiol (CBD). Both act on endocannabinoid system and are physiological regulators present in all mammals. Also, have several therapeutic properties, such as appetite stimulant, antiemetic, antitumor function, analgesia, anti-inflammatory, anxiolytic, antipsychotic and anticonvulsant [59–61]. However, due to factors such as side effects, societal stigma and legislation, its medicinal use remains limited [59].

The main cannabinoid is THC, followed by CBD, which makes up about 40% of the plant composition. Both principles act on endocannabinoid system and have therapeutic effects. However, in contrast to THC, CBD is free of psychotic effects and risk of dependence [62].

The active components of marijuana are reported to activate osteogenesis when they act on cannabinoid receptors CB1 and CB2, mimicking endogenous cannabinoids action. The CB1 receptor is present mainly in skeletal sympathetic nerve endings, regulating the adrenergic tonic restriction of bone formation that occurs after trauma. CB2 is expressed in osteoblasts and osteoclasts, stimulating production and inhibiting advanced bone resorption, and may be indicated in fractures due to decreased bone density [63].

Kogan et al. [64] produced bone fractures in rat femur and evaluated CBD effect. They found that stimulation of phytocannabinoid-induced bone repair process occurred during the healing stages (about 6 weeks). In contrast, Napimoga et al. [65] demonstrated that inhalation of Cs smoke inhibited early stages of bone healing around rat implants. The possible explanation for this disagreement is based on intramembranous healing pattern that occurs around the implants, where initial stages are the formation of a blood clot and/or its organization through primary bone formation. This process may be susceptible to the deleterious effects of Cannabis through CB1

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receptors expression in collagen fibers, which release norepinephrine, restricting bone formation and stimulating tissue resorption [66].

Ricinus communis

Castor bean (*Ricinus communis L.*) is a plant originating in southern Asia, but currently has a wide distribution, especially in tropical and subtropical regions. It is a dicotyledon belonging to the Euphorbiaceae family, characterized by seeds presence, rich in oil (48% to 50%), but toxic mainly due to a protein called ricin. However, its leaves have a lower concentration of this toxin. It is reported that the plant has antioxidant, anti-implantation, anti-inflammatory, antidiabetic, central analgesic, antitumor, larvicide, antinociceptive and anti-asthmatic activities. All these uses are due to the presence of certain plant constituents. The main compounds reported in this plant are rutin, gentisic acid, quercetin, gallic acid, kaempferol-3-O-beta-d-rutinoside, kaempferol-3-O-beta-d-xylopyranide, tannins, ricin A, B and C, castor agglutinin, indole-3-acetic acid and an alkaloid ricinin [67,68].

Castor oil extracted from *R. communis* fruit is used to obtain a polymer known as polyurethane with great properties. Plant polymers have been the target of many current researches in bone tissue engineering, searching for biomaterials to be used as growth inducers and molecule carriers that will contribute to the healing of large bone defects, such as grafts. These polymers have the advantage of being flexible in their processing and formulation, having excellent structural properties that do not emit toxic or irritating vapors and are biocompatible, being able to promote a slower resorption process, serving as bone cement for osteogenic bone conduction [69].

Polyurethane (CPR) found in the *Ricinus communis* plant has been studied for its biocompatibility and ability to stimulate bone regeneration. The effectiveness evaluation of castor bean polyurethane polymer with calcium carbonate by Nóbrega et al. [70] for use in unicortical ostectomy on dorsal face of third equine metacarpal by histological and immunohistochemical techniques, showed osteoconductive activity of biomaterial, confirmed by the presence of osteoblasts in biopsy specimens. Also, absence of chronic inflammatory response or foreign body reaction indicated biocompatibility. The osteoblast markers expression was detected in the newly formed tissue, so the results indicated that calcium carbonate castor polyurethane polymer may be an acceptable compound for use as bone substitute, in horses with fractures where bone filling is required. Del Carlo et al. [71] found that castor bean polymer, when added with calcium, enables osteogenesis and osteoconduction to occur, especially when stem cells are added, that allows the migration of capillaries, perivascular tissues and osteoprogenitor cells.

Ulmus wallichiana

Ulmus wallichiana Planch is an important South Himalaya plant species used in bone fractures treatment in both animals and humans [72]. One of its main discovered assets is naringenin-C-glucoside (NCG), a narigenin type. Swarnkar et al. [45] in order to study bone differentiation promoted by naringenin, the authors demonstrated the potency of this compound in osteoblast induction and differentiation in osteoblast cultures extracted from rat calvaria. Efficacy in vivo was evaluated by bone microarchitecture of rat calvaria using computed tomography and determination of new bone formation by fluorescent marking of bone. The second compound of this species, 2S, 3S-2,3 dihydroquercetin-C-glucoside, a new quercetin analog (Q) [73], through osteoblast culture and in vivo assays promoted peak of bone mass growth in first experimental group and preserved trabecular bone mass and cortical bone strength in the second experimental group, stimulating osteoblast proliferation, survival and differentiation.

Bixa orellanais

Bixa orellanais L is a native plant of Brazil but grows in other regions of South and Central America. It is grown in tropical countries such as Peru, Mexico, Ecuador, Indonesia, India, Kenya and East Africa. Annatto seeds are used as condiment, as well as laxatives, cardiotonics, hypotension, expectorants and antibiotics. It has anti-inflammatory activity for bruises and wounds and has been

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used for treating bronchitis. Leaf infusion has been shown to be effective against bronchitis, sore throat and eye inflammation [74]. Has in its chemical composition amino acids (tryptophan, methionine and lysine), carotenoids (bixin and norbixin), besides high fatty acid content and small amounts of linoleic and oleic acid [75].

Alves et al. [76] evaluated therapeutic effect of photobiomodulation (PBM) laser (780 nm) and polystyrene membrane coated with norbixin and collagen (PSNC) on bone healing in rats with calvarial bone defect. They were used to evaluate bone repair process. Histological staining, raman spectroscopy and scanning electron microscopy (SEM) were made. The PSNC membrane was effective in reducing the inflammatory process and served as support for bone repair. PBM laser also had positive effects on bone repair process with increased deposition and organization of newly formed bone. The PSNC membrane used in the study reduced the inflammatory infiltrate, which sustained the anti-inflammatory action of carotenoids present in annatto seeds [77]. Studies using animal models have shown that different types of biomaterials with or without use of PBM laser increased bone consolidation [78,79].

Pueraria Lobata

Puerariae root, known as Ge Gen, comes from *Pueraria lobata* root, is a traditional medicine commonly used to decrease bone density loss [80], as well as treat fever and cardiovascular disease, promote analgesia such as muscle relaxant [81]. Puerarina is one of the main phytoestrogens isolated from Gegen root (*Pueraria Lobata* Willd.), and when studied in a bone defect healing model in mice, it acted as a potent osteogenic agent, proving to be safe and ideal in bone defect repair [82].

Zang et al. [83] studied the in vitro behavior of osteoblasts in the presence of puerarin in various concentrations, in addition to performing osteoblastic implants in rats treated and untreated with puerarin for osteogenesis evaluation. The results showed higher levels of alkaline phosphatase and higher osteoblasts viability when compared to untreated groups. Also, a higher rate of bone formation in animals receiving osteoblastic implants was observed, demonstrating that it can affect the proliferation and differentiation of osteoblasts.

Corroborating these data, a rat model study of calvary defect using a combination of collagen structure and herbal extracts, including *Pueraria lobata* and *Salvia miltiorrhiza*, demonstrated that these species accelerated bone formation when used separately in combination to the collagen matrix [84].

Radix salvia miltiorrhiza

Danshen or *Radix salvia miltiorrhiza* is another well-known and widespread medicinal plant. One study examined Danshen's effectiveness in promoting healing of bone fractures and has shown to increase bone neoformation locally, in fractures experimentally induced in rabbit parietal bone. In addition, it can be used as a bone graft, especially in cases with compromised vascular responses, increasing the local vascular response [85].

Salvianolic Acid B (ASB), a very active ingredient found in the *Radix salvia miltiorrhiza* plant, has been reported as a potent anabolic agent. So, when studied against glucocorticoid-induced osteoporosis, it has improved the healing of osteoporotic fractures [85]. It was found that ASB and its analogs could increase angiogenesis, reduce bone marrow adipogenesis and proliferate osteocytes and lacunar canaliculi. This, in turn, would increase the volume of blood vessels to provide bone nutrition and could be used as a bone graft, especially in cases with compromised vascular responses [86].

Peperomia pellucida

*Peperomia pellucida (*L.) belongs to the Piperaceae family. It is a herbaceous plant found mainly in America, Africa and Asia. The species develops during rainy periods and in moist soils [87]. Traditional use includes treating abdominal pain, abscesses, acne, boils, cramps, fatigue, gout and rheumatic pains in the joints [88].

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Florence et al. [89] evaluated the effects of *P. pellucida* aqueous extract on fracture healing in Wistar rats by an experimental lesion on the femoral stem. The *P. pellucida* plant has been shown to have osteogenic compounds, such as flavonoids and phytoestrogens [90], which have the ability to stimulate osteoblast recruitment and activity at injury site. Active osteoblasts, in turn, secrete large amounts of alkaline phosphatase and osteocalcin, which are important in bone mineralization, hence the calcium deposition. In addition, this medicinal plant contains calcium and flavonoid metabolites that are known to contribute in matrix deposition during osteogenesis and promote bone formation, respectively [89].

Chinese herbal medicine has developed modern research for thousands of years, demonstrating its therapeutic effects in various diseases treatment [91]. Many Chinese medicinal plants are used to treat musculoskeletal system injuries, but little is known about their chemical structures, which makes the lack of relevant evidence-based scientific support and good documentation of clinical data serious. Saponins, cinnamic acid, anthraquinones and iridoids are main active constituents of Chinese plants, but there are few studies on these metabolites and their respective bone repair pathways [92,93].

Plant-based products

Peng et al. [4] developed a new topical paste used for fracture healing, consisting of extracts of six herbs, *Radix Dipsaci, Ramulus Sambucus Williamsii, Rhizoma Notoginseng, Flos Carthami, Rhizoma Rhei and Fructus Gardeniae.* It was developed according to a classical theory of traditional chinese medicine. The paste effects on bone healing and its transdermal absorption were determined using a rabbit fracture model. Callus sizes, bone-specific alkaline phosphatase levels and biomechanical properties of healed bone were evaluated. Herbal paste significantly increased cell proliferation in UMR-106 and HUVEC cells and inhibited nitric oxide production in murine macrophages in a dose-dependent manner. Its important chemical components, such as perperaponin VI, ginsenoside Rg1 and emodine, have been shown to be positively acting in the respective in vitro studies. Herbal paste significantly improved bone healing in rabbit fracture model, as indicated by the increase in callus size at weeks 2-5, and elevations in bone-specific alkaline phosphatase activities at weeks 5-6. Mass spectrometric analysis also showed presence of important chemical components of herbal formula in plasma after 8 weeks of topical treatment.

Mecsina hemostopper (MHS) is a herbal extract made from herbal agents such as Glycyrrhiza glabra extract, Alpinia officinarum, Thymus serpyllum, Syzygium aromaticum, Hypericum perforatum, Vitis vinifera, Urtica angustifolia, Mentha arvensis. This leads to an erythrocyte aggregation by creating a protein network in the bleeding area and enables bleeding control [94]. Some studies have been directing the applicability of these plant-based products for osteoregeneration. A study on the effects of low-level laser therapy with Mecsina hemostopper (plant extract-based product) on alveolar bone healing in female rats showed the presence of more bone tissue in the group treated with herbal extract along with laser therapy in comparison to other groups [95]. Mecsina Hemostopper was found to be an effective agent in increasing cell proliferation and providing more qualified bone formation. The combination of Mecsina and xenogeneic bone graft was found to be one of the most effective augmentation options for critical-size defects in rats. Mecsina Hemostopper could be used to get more qualified bone formation clinically, but more clinical research is needed in the future [96].

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Table 2. Plant species with osteointegrating activity scientifically validated.

References	Active compounds	Scientific name	Family	Popular name	Plant part	Study Model	Route of use	action mechanism
[28,38,105]	Flavonoids and monoterpenes	*Dysphania ambrosioides*	Chenopodiaceae	Mastruz; St. Mary's Wort	Leaves	*In vivo*	Local/ Graft	Osteogenesis activation, increased collagen deposition and bone alkaline phosphatase levels regulation.
[41,47,103,107–109]	Flavonoid (Naringenin)	*Piper sarmentosum*	Piperaceae	Daun Kadok	Leaves	*In vivo*	Oral	ROS reduction and oxidative stress prevention during the bone healing process. Acts on bone estrogenic receptors, stimulating bone mineralization and increased endochondral ossification.
[56–58,120]	Ascorbic acid, carotene A, ketosteroid, calcium, triterpenoids and β-sitosterol	*Cissus quadrangularis*	Vitaceae	Hadjod	Leaves	*In vivo*/ clinical	Oral	Stimulating the proliferation and differentiation of MSCs, as well as promoting bone neoformation through the Wnt/LRP5-B-Creatin signaling pathway for the pre-osteoblast's formation. Performance in bone estrogenic receptors stimulating ossification and early remodeling. Stimulates bone metabolism by increasing the uptake of calcium, sulfur and strontium minerals by osteoblasts in fracture healing.
[64,111]			Moraceae	Marijuana	Leaves		Culture cell	

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	Cannabidiol, Δ9-tetrahydrocannabinol	*Cannabis Sativa*				*In vitro and in vivo*	Local/graft Intraperitoneal	Stimulating the mRNA expression of enzyme Plod1 and Plod2 in primary osteoblast cultures, which in turn is involved in cross-linking and stabilization of collagenous extracellular matrix. They mimic endocannabidioids, activating CB2 receptors, expressed by osteoblasts and osteoclasts, thus attenuating bone loss induced by estrogen depletion due to osteoclasts suppression by suppressing RANKL expression.
[70,71,112,113]	Ricinoleic Acid (Polyurethane)	*Ricinus communis*	Euphorbiaceae	Palma Christi, tick, castor, tartago	Seeds	*In vivo*	Local/Graft	Polyurethane promotes fibroblast neoformation, progressively replacing the bone inside and around the biomaterial pores, in absence of late inflammatory reaction. Increased RUNX-2 expression and greater differentiation of osteoblasts.
[45,73]	Flavonoids (quercetin and naringenin).	*Ulmus wallichiana*	Ulmaceae	Chamormou	Stem bark	*In vitro* and *in vivo*	Culture cell Local/graft Oral	Anabolic effect and AhR transactivation. It mediates the proliferation,

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								differentiation and survival of osteoblasts and bone marrow cells by acting on estrogen receptors.
[76]	Norbixin (polystyrene)	*Bixa orellana*	Bixaceae	Urucum	Seeds	*In vivo*	Local/graft	Osteoconductive activity, stimulating bone cells through adequate surface roughness, proving to be efficient in osteoblasts proliferation.
[85,114–117]	Flavonoids (salvianolic acid B)	*Radix salvia miltiorrhiza*	Lamiaceae	Danshen	isolated compound	*In vitro* *in vivo*	Culture cell Local/graft Local/graft intralesional	Bone remodeling by gene expression of alkaline phosphatase, osteocalcin, osteoprotegerin and RANKL. Increased angiogenesis by positively regulating VEGF and VEGF-R2 gene expression. Stimulates osteogenesis through direct/indirect regulation of RANKL/OPG, BMP, Wnt/β-catenin, FoxO-3 and KLF1517 signaling pathways.
[83,84]	Isoflavonoids (Puerarina)	*Pueraria lobata*	Fabaceae	Gegen	Root	*In vitro* e *in vivo*	Culture cell Local/graft	Increased VEGF mRNA expression and increased alkaline phosphatase activity related to osteoblastic activity.

[4,93]	Asperosaponin VI and oleanolic acid Ginsenoside Rg1 and Rb1 Yellow hydroxyisaffl or A Kaempferol Emodina Rhein Geniposide * Not detected	*Radix Dipsaci Rhizoma Notoginseng Flos Carthami Rhizoma Rhei Fructus Gardeniae Ramulus Sambucus Williamsii*	Dipsacaceae Caprifoliaceae Araliaceae Compositae Polygonaceae Rubiaceae	Xu Duan Gui Zhi Hong Hua Da huang Zhi Zi * Not detected	Leaves and root	*In vitro* and *in vivo*	Culture cell Topic	Activation of osteogenesis. Nitric oxide inhibition, angiogenesis promotion and bone cell lines proliferation. Increased expression of alkaline phosphatase and osteocalcin. Anti-inflammatory activity, suppressing the production of NO and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6).
[89]	Minerals (calcium, phosphorus, magnesium, sodium and potassium) flavonoids	*Peperomia pellucida*	Piperaceae	Tortoise herb, little heart, tangon-tangon, peperomia, garrapatilla.	Whole plant	*In vivo*	Oral	Contributes to osteoid mineralization during bone formation, accelerates the migration and proliferation of osteogenic cells, increasing the quantity and activity of osteoblasts.
[95,96]	Terpenes, phenolic compounds, carotenoids, Isoflavones, isoliquiritin, diarylheptanoids and kaempferide, thymol, carvacrol, hyperforin, Menthol	*Glycyrrhiza glabra Alpinia officinarum Thymus serpyllum Syzygium aromaticum Hypericum perforatum Vitis vinifera Urtica angustifolia Mentha arvensis*	Fabaceae Zingiberaceae Lamiaceae Myrtaceae Hypericaceae Vitaceae Urticaceae Lamiaceae	Mecsina hemostopper	Whole plant	*In vitro and In vivo*	Culture cell Local/graft intralesional	Histologic assessment showed significantly more calcified tissue areas and significantly more osteoblast cells, was found to be an effective agent in increasing cell proliferation and providing more qualified bone formation.

Conclusion

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The use of medicinal plants as method to accelerate bone healing have been shown to be effective in tissue regeneration from the studies raised. The traditional use of plants brought by the Asian, African and South American communities has been extremely important as a starting point for medicinal herbs studies, as well as serving as an immediate treatment for pain relief and bone lesions healing. Scientific studies have confirmed the traditional use of medicinal plants obtained from ethnopharmacological surveys, showing that these biomaterials have curative, anti-inflammatory, antioxidant and osteogenic activities, capable of carrying out important signals for bone cell recruitment. Thus, medicinal plants are an important source in the search for new biomaterials to be implemented in medical practice. It is essential to deepen the study of various molecules made available by nature, to further elucidate action mechanism and development of regenerative therapies that improve patient care and alleviate conventional treatmen cost.

Conflict of Interest: The authors do not report conflicts of interest.

Abbreviations

ROS - reactive oxygen species
MSCs - mesenchymal stem cells
Wnt/LRP5-B – Wnt signaling pathway associated with lipoprotein receptor-related protein 5-beta
FoxO-3 signaling pathway
Signaling pathway KLF1517
mRNA - messenger ribonucleic acid
Plod1 enzyme
CB – cannabidioids
RANKL - receptor activator of nuclear factor-kappa B ligand
RUNX-2 - transcription factor 2
AhR - aryl hydrocarbon receptor
VEGF - vascular endothelial growth factor
VEGF-R2 - vascular endothelial growth factor receptor 2
OPG – osteoprotegerin
BMP - bone morphogenetic protein;
NO - nitric oxide
TNF-α - tumor necrosis factor-α
IL-1β - interleukin 1-beta
IL-6/11/18 - interleukin-6,11,18
HIV
RENISUS - National Register of National Health System Interest Plants
ER - estrogen receptor
Ps - Piper sarmentosum
H_2O_2 - hydrogen peroxide
MSC - mesenchymal stem cells
Cs - Cannabis sativa
THC - Tetrahydrocannabinol
CBD - Cannabidiol
CPR - Polyurethane
NCG - naringenin-C-glucoside
Q - quercetin analog
PBM - photobiomodulation
PSNC - polystyrene membrane norbixin and collagen
SEM - scanning electron microscopy
ASB - Salvianolic Acid B
UMR-106 Osteoblast-like cell culture
HUVEC - human umbilical vein endothelial cells

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