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Natural compounds in the treatment of osteosarcoma: just chemotherapy adjuvants? or is there much more?

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Abstract
Osteosarcoma is the most common type of bone tumor. Different therapeutic approaches are used in the management of osteosarcoma, where surgery is most often performed, often preceded and followed by chemotherapy, which however has serious short- and long-term side effects. Recently, much attention has been paid to natural compounds that can determine cell death of tumor. Some have a direct cytotoxic effect, and they also can reduce tumor and metastatic activity. Furthermore, it described how these molecules can also interact with elective chemotherapy against osteosarcoma. In this review, we have analysed the last decade literature, highlighting both the studies that have a direct cytotoxic effect on osteosarcoma, and those that are used as adjuvants to chemotherapy, allowing to reduce treatment doses, limiting the side effects. The comparison between the different studies have led to relevant considerations on the study standardization.

Keywords Osteosarcoma, Nutrigenomics, Natural compound, Chemotherapy

Introduction

Osteosarcoma (OS) is one of the most common malignant bone tumors, consisting of primitive mesenchymal cells involved in bone formation i.e. osteoid-producing cells, a non-mineralized bone matrix [1]. OS has a bimodal distribution referring to two different age groups: a first peak during adolescence with a significant age of outbreak between 10 and 14 years and a second peak in adults with an age above 65 years, in which it very often arises as a secondary neoplasm [2]. The annual incidence of osteosarcoma is approximately of two to four cases per million persons worldwide, whilst the International Incidence of Childhood Cancer reported that the total estimated number of cases per year in Europe across all age groups is 1135, with a peak incidence of 5 cases per 1.000.000 between 15 and 24 years [3, 4].

The most common symptom is constant pain that does not tend to regress with the use of analgesics, sometimes a palpable mass may be associated with localized pain. Among other systemic symptoms, the most frequently reported are general illness, flue and somnolence [5].

OS consists of a bone microenvironment with different cellular subpopulations including bone cells (osteoblasts, osteocytes and osteoclasts,), stromal cells (mesenchymal stem cells and fibroblasts), vascular cells (endothelial cells

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and pericytes), immune cells (macrophages and lymphocytes) and a mineralized extracellular matrix (ECM). In this complex and dynamic environment, cancer stem-like cells (CSCs) are a small subset of tumor cells that seems to be responsible for drug resistance, tumor recurrence, propagation and metastasis due to their self-renewal capacity and peculiar adaptability. Genomic instability, epigenetic alterations and metabolic plasticity play a role in the formation and progression of cancer stem-like cells. Due to their capacity to renew themselves to induce resistance this explains why cancer can still recur after initial successful treatment [5, 6].

OS is characterized by rapid cell growth and formation of metastases contributing to making this tumor highly aggressive and capable of causing pathological damage to bone tissue [6]. Since 80% of OS patients develop non-local metastases in other organs, improving patient survival rate and the efficacy of treatments in counteracting cellular and metastatic proliferation remains a fundamental issue to be addressed [7].

The standard treatment for osteosarcoma to date is the administration of chemotherapeutics, often compounded together. The aimed chemotherapeutics currently in use are doxorubicin, cisplatin, ifosfamide and high-dose methotrexate. The combined regimen of polychemotherapy and surgery remains largely not enough due to multidrug resistance [8].

In recent years numerous studies have demonstrated that the tumor microenvironment (TME) seems to influence the clinical course and therapeutic efficacy by modulating tumor chemoresistance [9, 10]. The use of high-dose chemotherapeutics also causes significant systemic side effects in patients such as cardiac toxicity, nephrotoxicity, neurotoxicity, hearing loss, infertility, immunosuppression and second malignancies, well exposed in next paragraph [11–54].

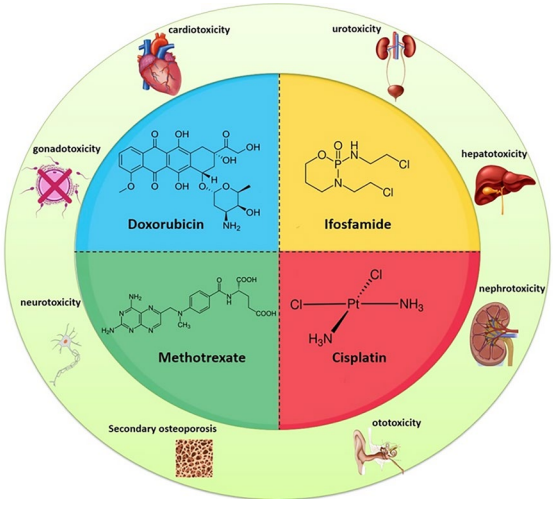

Fig. 1 schematic draw of principal chemotherapeutic molecules and possible induced side effects

Side effects of chemotherapeutic molecules used in osteosarcoma: mechanism of actions

OS is the most common bone tumor in children and young adults. Outcomes have improved, with median survival for non-metastatic disease reaching ~ 70% using pre- and post-operative chemotherapy. Standard agents are doxorubicin (Adriamycin, DOX), cisplatin (cis diamminedichloroplatinum II, CDDP), ifosfamide (IFO), and high dose methotrexate (HDMTX), used alone or in combination. These drugs cause important short- and long-term toxicities, including cardiotoxicity, nephrotoxicity, neurotoxicity, infertility, secondary osteoporosis and second malignancies, whose management is a key component of care. Reducing treatment related complications by identifying agents that prevent or attenuate side effects is therefore a major goal (see Fig. 1; Table 1).

Doxorubicin

Doxorubicin (DOX) is an anthracycline widely used for its broad antitumor activity. Its main dose limiting side effect is cardiotoxicity, but it can also induce nephrotoxicity, neurotoxicity, infertility, secondary osteoporosis and second malignancies. A prospective randomized trial showed that cardiotoxicity increases with cumulative DOX dose [12]. Cardiac damage may appear years after therapy and contributes substantially to non-cancer morbidity and mortality. The mechanisms are multifactorial and include marked oxidative stress, mitochondrial dysfunction in cardiomyocytes, and disruption of cardiac bioenergetics. Cardiac troponins and brain natriuretic peptide can help detect early cardiotoxicity [13]. Systemic intravenous DOX reaches all tissues and is cleared slowly by liver and kidneys, leading to frequent hepatic and renal lesions. In the kidney, DOX damages the nephron, especially glomerular podocytes, inducing apoptosis and epithelial–mesenchymal transition (EMT), which promotes renal fibrosis. DOX induced nephrotic syndrome presents with hypoalbuminemia, proteinuria, hyperlipidaemia and edema [14]. Although DOX was long considered unable to cross the blood–brain barrier, many preclinical studies have reported neurotoxicity. In humans, this is reflected in cognitive dysfunction and depressive symptoms. A meta-analysis of breast cancer patients treated with DOX showed significant deterioration in global cognition compared with controls, particularly in executive function, language, memory, verbal short-term memory and processing speed, while other domains remained unchanged [15, 16]. Few, mostly older, data are available on peripheral neurotoxicity. In vivo rat studies have shown degeneration and loss of ganglion cells in spinal, paravertebral and trigeminal ganglia, likely

Table 1 List of chemotherapeutic agents used in osteosarcoma treatment

Chemotherapeutic agent	Acronym	Dose (Maximum cumulative dose:)	Administration in osteosarcoma	Main side effects considered during and after administration	other possible side effects
Doxoruubicin	DOX	in combination: ~75 mg/m^2 for cycle (adult: 450 mg/m^2)	intravenous	cardiac toxicity, and myelosuppression	nephrotoxicity, neurotoxicity, infertility, secondary osteoporosis and second malignancies
cis-diamminedichloroplatinum II (Cis-platin)	CDDP	in combination: ~100 mg/m^2 for cycle (adult: 480 mg/m^2)	intravenous	Nephrotoxicity and ototoxicity	cardiac toxicity, neurotoxicity, infertility, secondary osteoporosis and second malignancies
Ifosfamide	IFO	in combination: : ~3,75 g/m^2 (standard dose) ~12 g/m^2 (high dose) for cycle (adult: 90 g/m^2)	intravenous	Nephrotoxicity, urotoxicity and infertility	cardiac toxicity, neurotoxicity, secondary osteoporosis and second malignancies
High dose of Methotrexate	HDMTX	in combination: ~12 g/m^2 for cycle (adult: 144 g/m^2)	intravenous	Epatotoxicity and nephrotoxicity	cardiac toxicity, nephrotoxicity, neurotoxicity, infertility, secondary osteoporosis and second malignancies

due to drug penetration through the porous vasculature of these structures [17, 18]. DOX caused significant loss of sensory neurons, and remaining neurons displayed marked morphological abnormalities consistent with degeneration [19]. Similar ganglionopathy was induced in rabbits in a dose-dependent manner and in rhesus monkeys after prolonged treatment (10 months) [20]. Because many cancer patients already suffer from depressive disorders, better characterization of DOX related neurotoxicity and its mechanisms is needed [16]. DOX also causes loss of primordial follicles, premature ovarian failure and infertility, affecting many young female cancer survivors. Chemotherapy alters the ovarian stroma and vasculature, reducing blood supply and increasing oxidative stress. This leads to a marked reduction in primordial, primary, preantral and Graafian follicles, thinning of the uterus and myometrium, and thickening of the endometrial gland, all associated with reduced endometrial receptivity and infertility [21]. DOX induced secondary osteoporosis appears to result from oxidative stress, which enhances osteoclast differentiation by upregulating regulatory factors (Rank, Nfatc1) and effector genes (Trap, Ctsk) [22], while impairing osteogenic differentiation via downregulation of transcription factors such as Osterix [23], ultimately causing bone loss.

Cisplatin

Cisplatin (cis-diamminedichloroplatinum-(II), CDDP) is another key chemotherapeutic agent. Its main toxicities include cardiotoxicity, nephrotoxicity, neurotoxicity, ototoxicity and gonado-toxicity, with nephrotoxicity being dose limiting [24–26]. CDDP induced renal injury involves pyroptosis, oxidative stress and inflammation [24], which activate several programmed cell death pathways (e.g., apoptosis, pyroptosis) [24, 27–30]. Because of this multifactorial pathogenesis, strategies targeting only inflammation or oxidative stress provide limited protection [31]. Cardiac complications from CDDP resemble those of DOX and involve disruption of ionic homeostasis, impaired mitochondrial respiration, oxidative stress, inflammation and apoptosis. These changes are reflected by increased serum creatine kinase, creatine kinase MB, lactate dehydrogenase and plasma cardiac troponin I [32]. CDDP causes peripheral neurotoxicity through nuclear and mitochondrial DNA damage in neurons. The high susceptibility of dorsal root ganglia relates to the lack of a blood–brain barrier, low glutathione levels and expression of specific organic cation transporters. CDDP forms DNA Pt adducts in both nuclear and mitochondrial DNA; accumulation of these adducts leads to axonal damage, neuronal degeneration and apoptosis [26]. CDDP ototoxicity results from apoptosis of sensory cells in the organ of Corti, damage to the spiral ganglion (myelin sheath detachment) and injury to the stria vascularis (edema, swelling, rupture). ROS generation exhausts cochlear antioxidant defenses, triggering inflammation and activation of apoptotic pathways [33, 34]. Ototoxicity is dose dependent and generally irreversible, so audiometry is recommended at baseline and during treatment. In cases of severe ototoxicity, CDDP should be permanently discontinued [35, 36]. Like DOX, CDDP causes permanent loss of primordial follicles. Damage to granulosa cells, which are essential for hormone production and follicular maturation, leads to hormonal imbalance, ovarian failure and infertility [21]. In males, CDDP has a gonadotoxic effect, causing a marked decrease in germ cells and spermatogonia and reduced germ cell proliferation. These alterations can persist for weeks, which is especially relevant in paediatric, pre- and post-pubertal patients [37]. CDDP induced oxidative stress also reduces bone mass, causing secondary osteoporosis. This is mainly due to decreased osteoblast activity and thinning of the growth plate, resulting in reduced bone formation and osteopenia, which can be partially reversed by reducing agents [38].

Ifosfamide

Ifosfamide (IFO) is an oxazaphosphorine alkylating agent with broad antitumor activity. It is a prodrug metabolized mainly by hepatic cytochrome P450 oxidases to several active species, including isophosphoramide mustard (IPM), acrolein, dechloroethylated compounds, chloroacetaldehyde (CAA) and thiodiaglycolic acid (TDGA). These metabolites underlie a wide range of toxicities—hepatotoxicity, nephrotoxicity, cardiotoxicity, neurotoxicity, metabolic disturbances and gonadal toxicity—with nephrotoxicity being the major dose limiting effect [39]. Liver toxicity is closely linked to prodrug activation and is characterized by hepatocyte necrosis, architectural disruption, micro-vesicular steatosis with vacuole formation and inflammatory infiltration in portal tracts [39]. Nephrotoxicity may affect both glomeruli and tubules and correlates with cumulative dose. It may present as isolated tubular dysfunction or full Fanconi syndrome with phosphaturia, glucosuria, aminoaciduria and tubular acidosis. Renal toxicity often appears during treatment but may also emerge months or years after therapy. Co administration of other nephrotoxic chemotherapeutics or radiotherapy increases the risk of long-term renal dysfunction. Interindividual variability in IFO nephrotoxicity is substantial and relates to differential production and inactivation of toxic metabolites, for example via aldehyde dehydrogenase (ALDH) [40].

IFO does not directly damage cardiomyocytes [41], but acute, reversible arrhythmias and contractility changes suggesting ischemia or injury have been reported [42]. Regarding neurotoxicity, recent work has refined the underlying mechanisms. Neurological damage was

initially attributed to 2 chloroacetaldehyde, but a clinical study by Beyoğlu et al. showed that its levels did not correlate with neuropathy. Instead, neurotoxicity was associated with inhibition of O phosphoserine phosphohydratase (impairing conversion of 3 phospho-serine to serine), impaired degradation of ceramides into cerebrosides, and oxidative damage requiring high glutathione (GSH) synthesis [43].

Data on IFO gonado-toxicity are limited and inconclusive, partly because many patients decline fertility assessment and because IFO is often given with known gonadotoxic drugs such as DOX or CDDP. Men appear more susceptible than women, but in females, ovarian dysfunction requiring hormone replacement therapy has been observed; other reports describe transient dysfunction with partial or complete recovery [44]. IFO also increases the risk of severe urotoxicity, including haemorrhagic cystitis. An analysis of the FAERS database by Gu and Samarneh revealed heterogeneous urotoxic events and presentations, though these findings require confirmation in clinical studies because of selection bias and incomplete data (e.g., dose, severity) [45]. As an alkylating agent, IFO may cause hypogonadism and consequent reduction in bone mineral density, worsened by hypophosphatemia from nephrotoxicity [46, 47].

High-dose methotrexate

Methotrexate (MTX) is a folate antimetabolite that reversibly inhibits dihydrofolate reductase. When administered at ≥ 500 mg/m² it is defined as high dose methotrexate (HDMTX) and can cause severe toxicity. Approximately 90% of MTX is excreted by the kidneys, where MTX and its metabolite 7-hydroxymethotrexate can precipitate in the acidic tubular environment, leading to crystalluria and nephrotoxicity. Impaired renal function delays MTX clearance, prolonging exposure to high concentrations and increasing the risk of other adverse events [48].

MTX associated hepatotoxicity is closely linked to excess reactive oxygen species (ROS). Oxidative stress induces lipid peroxidation, disrupts membrane integrity and causes hepatocellular injury. Endoplasmic reticulum (ER) stress activates the unfolded protein response, initially reducing protein synthesis but ultimately triggering programmed cell death if the oxidative insult persists [49]. HDMTX cardiotoxicity is also driven by oxidative and nitrative stress, which alters nitric oxide (NO) metabolism and activates apoptosis via the p38/JNK pathway [50].

Neurotoxicity from HDMTX appears related to age, exposure level, hepatic dysfunction and altered MTX clearance. Genetic background also plays a role: Latino white children, who have a higher incidence of acute lymphoblastic leukemia, show increased risk of HDMTX related neurotoxicity compared with non-Latino white children, indicating that ethnic (genetic) differences may influence the risk–benefit balance of HDMTX [51, 52]. HDMTX induced oxidative stress can also increase infertility risk, particularly in paediatric patients. Recent studies suggest that antioxidant strategies may reduce gonadal damage, although their impact on antitumor efficacy remains unclear [53]. MTX related osteopathy and secondary osteoporosis are rare and poorly characterized, but appear to reflect a dose dependent residual effect on bone forming cells [54].

Clinical monitoring and long-term surveillances in OS chemotherapy

Although these chemotherapeutics share many toxicities, the specific side effects requiring intensive monitoring differ by drug. For DOX, the main concerns are cardiotoxicity and myelosuppression. Cardiac injury can be acute or delayed by years, so long term surveillance with echocardiography or radionuclide ventriculography (MUGA scan) is recommended, particularly in high-risk patients or those exceeding threshold cumulative doses [12, 13]. Myelosuppression, causing neutropenia, anaemia and thrombocytopenia, requires a complete blood count (CBC) at each treatment cycle and again 10–14 days after administration [55]. For CDDP, nephrotoxicity is the most common and dose-limiting side effect and can cause serious, sometimes irreversible renal damage [24, 27–30]. Renal function must therefore be carefully monitored before and during each cycle. Although ototoxicity does not affect survival, it markedly impairs quality of life, so regular audiometric evaluations are recommended [34, 36]. For IFO, surveillance focuses on nephrotoxicity and urotoxicity, with periodic assessment of renal function and use of uroprotective agents [40, 45]. Given the high risk of infertility, especially in paediatric, adolescent and reproductive-age patients, fertility preservation options should be discussed before initiating IFO [44]. In HDMTX therapy, monitoring of liver enzymes and renal function is essential to prevent liver fibrosis and cirrhosis (rare but serious) and to avoid renal failure, particularly in cases of delayed MTX clearance [48, 49].

Rationale for adjuvant strategies

The severity of these side effects complicates prognosis and survival, not only by worsening quality of life but also by increasing morbidity and mortality through the development of additional diseases. Strategies that allow reduction of chemotherapy doses while maintaining efficacy are therefore highly desirable. Recent studies have highlighted the cytotoxic activity of several natural compounds against tumor cells, suggesting that they could be used as adjuvants to enhance the efficacy of standard OS agents (DOX, CDDP, IFO, HDMTX), permitting

dose reduction and thereby mitigating treatment-related toxicities.

Natural compounds and osteosarcoma: what is the way?

Recently, natural molecules have emerged as potential therapeutic agents acting in cancer progression and metastasis development. Natural compounds are used thanks to their antitumor capacity, availability, and ability to overcome resistance of tumor cells, safety and efficacy. One of the most important sources of biologically active compounds is the plant kingdom. Currently, more than 3,000 species of plants have been accounted in the treatment of cancer and clinical trials so far [56]. All of these natural compounds can be categorized into different chemical types. These include flavonoids, terpenoids, phenolic acids, tannins, phenylpropanoids (like coumarins, lignans, monolignols), naphthoquinones, polyketides (like anthraquinones), stilbenes, curcuminoids, alkaloids and betalains [57, 58].

Flavonoids are secondary metabolites synthesized by plants in which they act by defending against pathogens and promoting their growth and development, but they are also abundantly found in commonly consumed fruits, vegetables, grains, and herbs. These compounds have demonstrated antioxidant, anti-inflammatory, differentiation and apoptotic effects by downregulating Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) and consequently proinflammatory cytokines, phosphorylation of Mitogen-Activated Protein Kinases (MAPKs), as well as having epigenetic effects on DNA methylation, histone modification and noncoding of RNA which may account for their potential therapeutic benefits in several orthopaedic diseases [59–61]. Among the secondary metabolites produced mainly by plants are also terpenoids, commonly named isoprenoids. As already found for flavonoids, terpenoids show high antioxidative activities through epigenetic regulation, present beneficial and protective activities for pathological bone erosion, osteoporosis development and has antiarthritic effects by alleviating bone erosion in rheumatoid arthritis by activating antioxidant enzyme and Nrf2/p62 signalling [62, 63]. Furthermore, other class of natural compounds, such as, phenolic acids, stilbenes, tannins and phenylpropanoids represent well-studied plant secondary metabolites, arising from the same biosynthetic pathway as flavonoids and that are associated with potent anticancer abilities in various in vitro and in vivo studies [64–66]. The therapeutic activities of phenolic acids are reinforced by their role as epigenetic regulator and to their antioxidant activity [67, 68]. It has been shown that phenylpropanoids, show antitumor effects depending on their effects on immune regulation, cell growth and differentiation with a caspase-dependent apoptosis mechanism modulating signal transduction pathways containing GTP-binding proteins and reducing Bcl-2 expression, and also has beneficial role in osteoarthritis and osteoporosis [69, 70]. Also, tannins have been shown to possess antioxidant activity via a mechanism involving the reduction of NAD(P)H oxidase activation and intracellular ROS generation, anti-inflammatory activity decreasing the release of the pro-inflammatory cytokine through the inhibition of the inflammatory NF-kB/TLR-4 pathway and related factors and anti-cancer activity exerting anti-proliferative and pro-apoptotic effects via a mechanism involving caspases-3 and 9 as well as PARP activation [68]. The outcome of naphthoquinones and polyketides (like anthraquinones), a type of quinone that are synthesize in nature where they have an important role in the metabolic cycles, reveals their important anticancer effect by inducing cell death and autophagy, through several mitogenic signalling pathways, including NF-kB, STAT3, and AKT. They also have pharmacological properties such as anti-inflammatory and analgesic effects [71]. Several natural alkaloids, which are polycyclic and nitrogen-containing compounds obtained from plants, have been successfully approved as chemotherapeutic drugs thanks to their anticancer activity against cell growth, cell cycle, cell invasion, angiogenesis, metastasis, autophagy and apoptosis, as well as their anti-osteoporotic properties by stimulate osteoblast proliferation and differentiation, and by downregulation of the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) [72, 73].

Natural compounds have been proven to exhibit chemotherapeutic and/or chemo-sensitizing effects on cancer cells, enhanced the effectiveness and tolerance of chemotherapeutic agents both when used in combination with chemotherapy and when used alone [69]. Some compounds enhance the antitumor effect when used in combination with chemotherapy and this reveal their role as a promising therapeutic adjuvant agent for OS cell treatment [74]. Most of them enhance the therapeutic effects of cancer chemotherapy either increasing the drug's bioavailability or blocking and modulating one or more targets in the signal transduction pathway which are implicated in promoting apoptosis, inhibiting cellular proliferation, and mobilizing the immune system [75]. Therefore, natural compounds act as chemotherapeutic adjuvants by sensitizing cancer cells to be more responsive to chemotherapeutic drugs and for this potentiation the tumoricidal effect. This effect is achieved through reversing chemoresistance by increase the accumulation of the drugs in cancer cell and promoting the repairing mechanism in normal cells against damage of chemotherapeutic drugs. Also, combination therapy reduces the toxicities associated with traditionally used

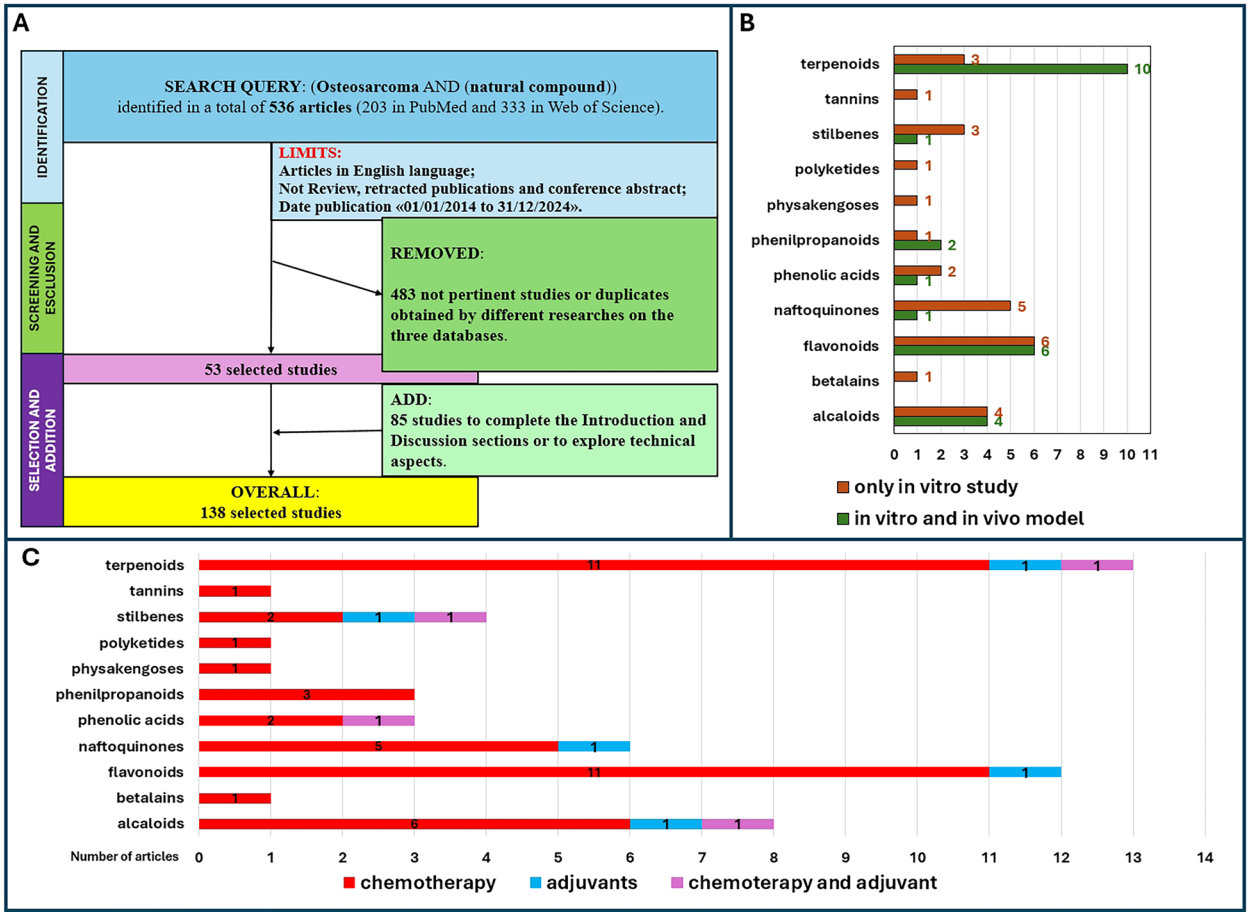

Fig. 2 Flowchart of the search strategy of the selection of bibliographic references (**A**) and of the different tables (**B**, **C**) that summarize some information obtained from the selected articles

chemotherapeutics [75, 76]. All these properties can be clustered under the name "Nutrigenomics Effects", being nutrigenomics the science that studies the intricate relationship between nutrition, natural compounds, gene expression, and health outcomes [77].

Currently, many natural anti-cancer drugs are in clinical use for chemotherapy such as taxanes (taxol), vinblastine, vincristine, due to their anticancer properties. They effectively suppress cell proliferation, regulates the cell cycle, and interferes with several tumorigenic signalling pathways, such as phosphoinositide 3-kinase (PI3K), matrix metalloproteinase (MMP), MAPK/ERK known as the Ras-Raf-MEK-ERK pathway, toll-like receptor (TLR) pathway, and AKT pathway and also play a role in DNA repair mechanisms [78].

The use of natural compounds in combination with cancer therapeutics play a significant role for their synergistic effects but a promising strategy would be to aim to use them as chemotherapeutics for the treatment of cancer [75, 78].

This systematic review provides the recent literature from the past ten years on isolated natural compounds and assesses their potential on osteosarcoma, both as chemotherapeutic compound and/or chemotherapeutic adjuvant.

Research strategy and results of collected references

The following literature research was carried out in two different databases: MEDLINE and Web of Science databases. The strings used in bibliographic research are: (Osteosarcoma AND Natural compounds). In the MEDLINE database (PubMed research engine) 204 articles were retrieved by considering publications written in English (AND "English" [language]) and published after January 1, 2015 (AND ("2014/01/01"[Date - Entrez]: "2024/12/31 "[Date - Entrez])). Reviews (NOT Review [Publication Type]) were then excluded, reducing the number of collected articles to 101. Similarly, in Web of Science databases, 105 articles were found respectively, using the same strings and limitations (years range, language, and research articles). Then, four reviewers manually assessed the title and abstract of collected references and those not considered pertinent (e.g. research articles

Table 2 List of natural compounds exhibit chemotherapeutic and/or chemo-sensitizing effects

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Saurolactam	**Alkaloids**	In vitro	MG63 cell line; HOS cell line;	20–40 μM	Akt, PI3k;	Promoted G1 cell cycle arrest; Promoted apoptosis; Inhibited proliferation, migration and invasion ability	Chemotherapy	[84]
		In vivo	Male Balb/c nude mice;	25 mg/kg/day of body weight (intraperitoneal injection)		Inhibited tumor growth		
Sanguinarine		In vitro	HOS cell line; U-2OS cell line;	0–1,6 μM	PI3K; Akt; mTOR; JAK; STAT3	Promoted G2/M cell cycle arrest; Promoted apoptosis; Increased ROS levels	Chemotherapy	[83]
		In vivo	Mice; Primary and metastatic tissues;	8 mg/kg/day (intraperitoneal injection)		Inhibited tumor growth		
Voacamine		In vitro	SAOS-2-WT cell line; SAOS-2-DX cell line; Me30966 cell line;	0.5–1.0 μg/mL	-	Exhibited a significant reduction of survival	Adjuvant	[136]
Piperlongumine		In vitro	MG-63 cell line; 143B cell line; KHOS/NP cell line;	8.8 μM for MG-63 cell line; 9.3 μM for 143B cell line; 9.6 μM for KHOS/NP cell line.	-	Promoted G2/M cell cycle arrest; Promoted Apoptosis; Increased ROS levels; Inhibited proliferation and migration ability; Induced chromatin condensation and nuclear fragmentation	Chemotherapy and adjuvant	[85]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
		In vitro	U2OS cell line; MG63 cell line; HFOB1.19 cell line; C28/I2 cell line;	2.5–10 μM	SOCS3/JAK2/STAT3	Inhibited proliferation; Promoted apoptosis; Reduced the miR-30d-5p expression; Inhibited cell invasion, migration, epithelial- mesenchymal transition	Chemotherapy	[81]
		In vivo	Tumor tissues in nude mice	2.5, 5 or 10 μmol/kg/day; (intraperitoneal injection)		Inhibited tumor cell growth		
		In vitro	MG-63 cell line; U2OS cell line;	0–17.5.5 μM	ROS/PI3K/Akt	Promoted G2/M cell cycle arrest; promoted apoptosis; Inhibited proliferation; Increased ROS levels;	Chemotherapy	[80]
Stylopine		In vitro	MG-63 cell line;	0.987–2.107 μM	VEGFR2	Inhibited proliferation and cell migration; Promoted apoptosis;	Chemotherapy	[79]
Cyclovirobuxine D		In vitro	143B cell line; HOS cell line;	20–50 μM	PI3K-AKT-mTOR	Inhibited proliferation; Inhibited migrative and invasive ability; Induced autophagy flux arrest; Induced lysosomal acidification;	Chemotherapy	[82]
		In vivo	BALB/c nude mice;	20, 40, 60 mg/kg (intragastrical gavage)		Inhibited tumor growth and lung metastasis.		
Betanin	**Betalains**	In vitro	MG-63 cell line;	9.08–54.49 μM	PI3K/AKT/mTOR/S6	Inhibited proliferation; Increased ROS levels; Promoted apoptosis; DNA damages; Inhibited cell migration.	Chemotherapy	[87]
Quercetin	**Flavonoids**	In vitro	143B cell line;	5–10 μM	PI3K/AKT	Inhibited proliferation; Enhanced cisplatin sensitivity of cells;	Adjuvant	[137]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
		In vitro	U2OS cell line;	0.01–100 μM	-	Promoted G2/M cell cycle arrest; Inhibited proliferation;	Chemotherapy	[96]
		In vitro	HOS cell line; MG63 cell line;	25, 50, 100 μM	-	Inhibited cell migration and invasion; Downregulated mRNA and protein expression;	Chemotherapy	[97]
		In vivo	BALB/c nude mice;	25, 50 and 100 mg/kg/day		Suppressed osteosarcoma cell metastasis		
		In vitro	MG63 cell line;	50, 100, 200 μM	-	Induced autophagic cell death; Increased accumulation of ROS;	Chemotherapy	[98]
		In vivo	BALB/c nude mice;	100 mg/kg		Inhibited tumor cell growth		
Acacetin		In vitro	143B cell line; MG63 cell line; SJSA cell line; HOS cell line;	15, 30, 45, 60 μM	JNK/c-Jun	Inhibited cell proliferation; Induced apoptosis; Increased ROS levels;	Chemotherapy	[90]
		In vitro	143B cell line; MG63 cell line; SaoS2 cell line; U2OS cell line;	30.15–28.09μM	Wnt; β-catenin; JNK	Promoted G2/M cell cycle arrest; Inhibited cell proliferation; Inhibited the migration and invasion ability;	Chemotherapy	[91]
		In vivo	Nude mice;	40,60,80 mg/kg (By gavage)		Inhibited the growth and metastasis;		
Hyperoside		In vitro	U2OS cell line; MG63 cell line;	150 μg/mL	TGF-b/BMP2	Promoted G0/G1 cell cycle arrest; Inhibited proliferation; Stimulated osteogenic differentiation;	Chemotherapy	[95]
Diosmetin		In vitro	Saos-2 cell line; U2SO cell line;	10, 30, 90 μM	STAT3; c-Myc	Promoted G2/M cell cycle arrest; promoted apoptosis; Inhibited proliferation;	Chemotherapy	[89]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Cinnamtannin		In vitro	143B cell line; MG63 cell line; U2OS cell line; HOS cell line;	10, 20, 40 μM	miR-1281/PPIF	Inhibited proliferation; Induced aberrant expression of miRNAs;	Chemotherapy	[99]
		In vivo	BALB/c nu/nu female mice	100 mg/kg/day (intragastrically)		Inhibited cell proliferation of tumor cells		
Casticin		In vitro	143B cell line; MG63 cell line;	0.4–2,8 μM	-	Promoted G2/M cell cycle arrest; Inhibited proliferation; Inhibited the migration and invasion ability; Induced ferroptosis	Chemotherapy	[94]
		In vivo	BALB/c nude mice	20, 40, 60 mg/kg (Intragastrically)		Inhibited tumor growth; Lower toxicity		
Licochalcone A		In vitro	143B cell line; MG63 cell line;	80, 40, 20, 10, 5, 2.5, 1.25, 0.6125 μM	-	Inhibited proliferation; Promoted apoptosis; Promoted autophagy; Inhibited the migration and invasion ability	Chemotherapy	[93]
Paucatalinone A		In vitro	HOS cell line; MG63 cell line;	5–10 μM	Mitochondria;/ERK1/2	Promoted G0/G1 cell cycle arrest; Inhibited proliferation; Promoted apoptosis; Inhibited colony and spheroid formation; Inhibited migration and invasion ability;	Chemotherapy	[92]
		In vivo	Old female BALB/c mice	5, 10 mg/kg (intravenous administration)		Inhibited tumor growth;		
Chimaphilin	**Naphthoquinones**	In vitro	U2OS cell line; KHOS cell line;	10 μM	PI3K; AKT	Inhibited proliferation; Promoted apoptosis; Increased doxorubicin-induced cell death;	Chemotherapy	[104]
		In vitro	U2OS cell line;	10, 20, 40 μmol/L	PI-3 K; Akt; ERK1/2; Smad	Inhibited proliferation; Inhibited cell migration; Inhibited cell metastasis;	Chemotherapy	[105]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Acetylshikonin		In vitro	U2OS cell line	5.68 μM	FOXO3	Inhibited proliferation; Promoted apoptosis; Inhibited migration ability; Increased ROS levels; Induced DNA damage;	Chemotherapy	[101]
Deoxyshikonin		In vitro	U2OS cell line; HOS cell line;	20 μM	P38	Inhibited proliferation; Promoted sub-G1 cell cycle arrest; Promoted apoptosis;	Chemotherapy	[102]
Shikonin		In vitro	Primary osteosarcoma cells; HOS cell line;	1–4 μm	EGR1-Bax	Inhibited proliferation; Inhibited the migration ability; Promoted apoptosis; Increased ROS levels;	Adjuvant	[106]
		In vivo	Nude mice;	5, 2.5 mg/kg/day (intraperitoneal injection)		Inhibited tumor growth		
Plumbagin		In vitro	MG63 cell line; U2OS cell line; HOS cell line;	0.59, 0.78, 1.24 μM	ER-stress	Reduced cell viability; Promoted apoptosis; Increased ROS levels; Increased intracellular calcium concentration;	Chemotherapy	[103]
Grifolic acid	**Phenolic acids**	In vitro	143B cell line; MG63 cell line; U2OS cell line; Saos2 cell line;	30 μmol/L	-	Inhibited proliferation; Decreased MMP; Inhibited ATP and NADH production;	Chemotherapy	[110]
		In vivo	Male nude mice;	0.4 ml, 0.1 mmol/L (intratumoral injections or tail vein injections		Induced necrosis; Inhibited the metastasis;		
Oleuropein		In vitro	MG63 cell line; Saos2 cell line;	50, 100, 200, 400 μM	-	Inhibited proliferation;	Chemotherapy	[107]
		In vitro	MG63 cell line;	20 μg/mL	-	Inhibited proliferation; Promoted autophagy; Induced metabolic imbalance;	Chemotherapy and adjuvant	[109]
Angedahurin	**Phenylpropanoids**	In vitro	MG63 cell line;	7.2 μM	-	Inhibited proliferation; Promoted apoptosis;	Chemotherapy	[112]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Anticarin-B		In vitro	MG63 cell line; U2OS cell line; Saos2 cell line;	0.5 µmol/L	-	Inhibited TRiC activity; Inhibited proliferation; Promoted apoptosis;	Chemotherapy	[113]
		In vivo	BALB/c nude mice;	5 mg/kg/day (intramuscular injection)		Inhibited tumor growth		
Geiparvarin		In vitro	HOS cell line;	0.7–1.8 µg/mL	-	Inhibited proliferation; Inhibited invasion and migration; Inhibited metastasis;	Chemotherapy	[114]
		In vivo	Female nude mice;	5 mg/kg/day (by IP injection)		Inhibited lung metastasis;		
Physakengose G	**Physakengoses**	In vitro	U2OS cell line; HOS cell line;	0–25 µM	EGFR/mTOR	Inhibited proliferation; Promoted apoptosis; Promoted lysosome and mitochondrial dysfunction; Inhibited autophagic flux;	Chemotherapy	[115]
Maclurin	**Polyketides**	In vitro	U2OS cell line; MG63 cell line;	0, 50, 100, 200 µM	PARP; p38-MAPK, ERK	Inhibited proliferation; Promoted apoptosis; Increased ROS levels; Inhibited the migration ability;	Chemotherapy	[117]
Resveratrol	**Stilbenes**	In vitro	MG63 cell line; Saos2 cell line; U2OS cell line; KHOS cell line;	60, 120 µM	PI3K; AKT	Inhibited proliferation; Promoted apoptosis; Promoted osteoblast differentiation; Inhibited cell migration; Sensitized cells to chemotherapy;	Chemotherapy and adjuvant	[73]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Oxyresveratrol		In vitro	Saos2 cell line;	0, 5, 15, 45 μM	STAT3	Inhibited proliferation; Promoted apoptosis;	Chemotherapy	[119]
Polydatin		In vitro	MG63 cell line; Saos2 cell line;	0–150μM	-	Inhibited proliferation; Promoted S phase cell cycle arrest; Promoted osteoblast differentiation and mineralization; Inhibited cell migration; Sensitized cells to ionizing radiation;	Adjuvant	[138]
Glaucocalyxin A		In vitro	143B cell line; SJSA cell line; HOS cell line; MG63 cell line;	0–20 μM	JAK2; STAT3	Inhibited proliferation; Inhibited colony formation; Promoted apoptosis; Promoted G2/M cell cycle arrest; Increased ROS levels;	Chemotherapy	[120]
		In vivo	female BABL/c nude mice	10, 20 mg/kg (Intraperitoneal injection)		Inhibited tumor growth;		
Urolithin B	**Tannins**	In vitro	MG63 cell line;	260 μM	-	Inhibited proliferation; Promoted apoptosis and necrosis; Promoted G2/M cell cycle arrest; Increased ROS levels; Inhibited the migration and invasion ability	Chemotherapy	[121]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Elemene	**Terpenoids**	In vitro	U2OS cell line; MG63 cell line;	10, 80, 160 μg/ml	RAS	Inhibited proliferation; Inhibited the migration and invasion ability; Promoted apoptosis	Chemotherapy	[133]
		In vivo	BALB/c nude mice	50 mg/kg (intraperitoneal injection)		Inhibited tumor growth		
		In vitro	MG63 cell line; U2OS cell line; Saos2 cell line;	0–25 μg/mL	-	Inhibited proliferation; Promoted apoptosis; Enhanced cytotoxic effect of doxorubicin; Induced the oxidative stress	Adjuvant	[134]
		In vivo	BALB/c nude mice	45 mg/kg/day (intraperitoneal injection)		Inhibited the tumorigenesis and angiogenesis		
Raddeanin A		In vitro	143B cell line; MG63 cell line; U2OS cell line; Saos2 cell line; HOS cell line;	1, 2, 4 μM	ROS; JNK; NF-κB	Promoted apoptosis; Inhibited migration and invasion ability; Suppressed NF-κB activation	Chemotherapy	[125]
		In vivo	Male BALB/c nude mice;	1.25 mg/kg, 2.5 mg/kg, 5 mg/kg/day (intraperitoneal injection)		Inhibited tumor growth		

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
		In vitro	MG63 cell line; U2OS cell line; HOS cell line;	1 μM, 2 μM, 4 μM	STAT3	Inhibited proliferation; Inhibited colony formation; Promoted apoptosis; Restored sensitivity to doxorubicin;	Chemotherapy and adjuvant	[127]
		In vivo	Female BALB/c nude mice;			Inhibited tumor growth		
		In vitro	143B cell line; SJSA cell line;	2 μmol/L	JNK; c-Jun	Inhibited proliferation; Promoted apoptosis; Increased ROS levels;	Chemotherapy	[126]
		In vivo	Male BALB/c athymic nude mice;	5–10 mg/kg		Promoted apoptosis; Inhibited tumor growth		
Bruceine D		In vitro	MG63 cell line; U2OS cell line; HOS cell line; Saos2 cell line;	0.1–20 μmol/L	STAT3	Inhibited proliferation; Promoted apoptosis; Inhibited cell cycle progression; Inhibited migration and invasion ability; Inhibited stem cell like properties and inhibited self-renewal ability	Chemotherapy	[129]
		In vivo	Female BALB/c nude mice;	2.5–5 mg/kg/day (intraperitoneally injection)		Inhibited tumor growth		
Ginsenoside RH2		In vitro	U2OS cell line;	8 μmol/L, 80 μmol/L	NF-κB; MAPK; PI3K; Akt; mTOR	Inhibited proliferation; Promoted apoptosis; Inhibited migration ability;	Chemotherapy	[131]
MO-OH - Nap		In vitro	143B cell line; MG63 cell line; Saos2 cell line; HOS cell line;	0.1 μM, 1 μM, 10 μM	UPR	Promoted apoptosis; Inhibited proliferation; Inhibited migration and invasion ability; Altered purine and pyrimidine metabolism; Deregulated iron homeostasis pathways;	Chemotherapy	[124]

Table 2 (continued)

Compound	Subgroup	Study type	Cells/ In vivo model	Doses	Identified Pathways	Effects of administration	Chemotherapy or adjuvant	Ref
Bruceantinol		In vitro	143B cell line; MG63 cell line; U2OS cell line; HOS cell line;	5–12.5 nM	STAT3	Inhibited proliferation; Inhibited STAT3 activities; Inhibited migration ability; Promoted apoptosis;	Chemotherapy	[130]
		In vivo	Mice;	2–4 mg/kg/day		Inhibited tumor growth		
Celastrol		In vitro	MG63 cell line; U2OS cell line; HOS cell line;	1 μM, 2.5 μM, 4 μM	Bcl2; BAX	Inhibited proliferation; Promoted apoptosis;	Chemotherapy	[123]
Degalactotigonin		In vitro	U2OS/MTX cell line; ZOS cell line; ZOS-M cell line;	40 umol/L	Hedgehog; Gli1	Inhibited proliferation; Promoted apoptosis; Promoted G2/M cell cycle arrest; Inhibited metastasis;	Chemotherapy	[132]
		In vivo	Athymic nude (nu/nu) mice;	150–300 mg/kg/day (intraperitoneal injection)		Inhibited tumor growth; Inhibited metastasis		
Hederoside C		In vitro	MG63 cell line; U2OS cell line;	1 μM, 5 μM, 10 μM	MAPKs; STAT3	Inhibited proliferation; Inhibited migration and invasion ability; Promoted apoptosis;	Chemotherapy	[128]
		In vivo	BALB/c athymic nude mice;	7,5 mg/kg/day (intraperitoneal injection)		Reduced tumor proliferation		
Dioscin		In vitro	MG63 cell line; Saos2 cell line;	IC50 value (not reported)	HuR; Pim1	Deregulated MiR-16-5p levels; Inhibited proliferation; Inhibited migration and invasion ability; Promoted apoptosis;	Chemotherapy	[135]
		In vivo	Tumor and para-tumor samples; BALB/c nude mice;	50 mg/kg/day (gavage administration)		Inhibited solid tumor growth;		

which not used isolated compounds) or retrieved duplicates were discarded. Then, 53 articles related to the topics of the review were selected. Finally, further 85 references were cited to add information on some technical aspects for a major understanding of mechanisms acting in cancer progression and metastasis development.

In Fig. 2, we have shown a detailed flowchart of the search strategies (Fig. 2A) and a summary of the results obtained from the selected studies by the literature researches (Fig. 2B and C). Then, the 53 selected articles were analysed by type of study, classes of molecules and whether the molecules were studied as chemotherapeutics, as adjuvants or both. All studies used in vitro models, but only 49% (26/53 articles) completed the study with an in vivo model. In Fig. 2B, all the classes of molecules identified are shown with the relative number of articles with only the in vitro study and the number of articles that also present the in vivo model. In Fig. 2C, we have highlighted how in the various classes of molecules all present the natural compound in its role in chemotherapy treatment, while very few evaluate its aspect as an adjuvant to chemotherapy. Table 1 lists all the selected articles where natural compounds were used in in vitro and in vivo studies, and the effects of its treatments in osteosarcoma (Table 2).

Natural compounds and osteosarcoma treatment: chemotherapeutic actions

Natural compounds represent an important resource in cancer therapy, as they show potent antitumor and antimetastatic activities and are being investigated to improve OS treatment.

Alkaloids

Alkaloids are a highly diverse class of natural secondary metabolites with broad pharmacological properties, including anticancer effects [79]. Among those studied in OS are stylopine, piperlongumine, cyclovirobuxine D, sanguinarine and saurolactam [80–86]. All, except cyclovirobuxine D, induce cell death mainly via mitochondrial apoptosis, evidenced by Annexin V (ANXV)/propidium iodide (PI) and ethidium bromide (EtBr)/Acridine orange (AO) staining. They act through the intrinsic pathway by downregulating anti-apoptotic proteins Bcl 2 and Bcl xL and upregulating Bax, which promotes cytochrome c release, caspase-9, 3 and 7 activation, and PARP cleavage, ultimately leading to apoptosis [80–82, 84, 85]. Cyclovirobuxine D instead triggers cell death by inducing autophagy, which is subsequently blocked at a late-stage due to excessive lysosomal acidification mediated by interaction with the A1-subunit of the V0 domain of V-ATPases (ATP6V0A1). This results in an increased LC3 II/LC3 I ratio and inhibition of p62 degradation, indicating impaired autophagic flux [83]. Several key oncogenic pathways are inhibited. Stylopine targets VEGFR2 signalling by reducing VEGFR2 expression and phosphorylation, thus inactivating downstream effectors [80]. Piperlongumine, cyclovirobuxine D, sanguinarine and saurolactam markedly reduce phosphorylated Akt, PI3K and mTOR and their downstream targets, including Skp2 and c Myc [81, 83–85]. Piperlongumine and sanguinarine also inhibit the JAK/STAT pathway, reducing phosphorylation of JAK1, JAK2 and STAT3. Piperlongumine further suppresses miR-30d-5p, a microRNA involved in tumor development [81, 84, 85]. Cell cycle arrest contributes to their antiproliferative action: piperlongumine and sanguinarine induce G2/M arrest with decreased cyclin B1 and cdc2 and increased p21 and p53, whereas saurolactam promotes G1 arrest [82, 84, 85]. Piperlongumine also downregulates migration related genes and inhibits cell migration [86]. These mechanisms have been confirmed by in vivo experiments, using xenograft OS models with intraperitoneal or intragastric administration, where treatment reduced tumor growth and metastasis consistent with in vitro findings [82–85].

Betalains

Betalains are pigments of about 17 families of plants as they are hydrophilic, they are accumulated in the vacuoles of the cells, and have been demonstrated to have antiviral, antibacterial, antioxidant, antiradical, antilipidemic and anticancer activities [87]. They comprise betanin, which has been shown to have anti-cancer and pro-apoptotic effects through the inhibition of PI3K/Akt/mTOR pathway, already described previously [88].

Flavonoids

Flavonoids are a large family of natural compounds with well-documented anticancer properties [89]. The flavonoids studied in osteosarcoma include diosmetin, acacetin, paucatalinone A, licochalcone A, quercetin, casticin, hyperoside and cinnamtannin [90–100]. Diosmetin, acacetin and paucatalinone A induce apoptosis, as shown by ANXV/PI staining, via upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins [90–93]. Licochalcone A and quercetin trigger autophagic cell death, increasing LC3-II and decreasing p62. Quercetin also upregulates Nuclear Protein 1 (NUPR1), which promotes the expression of autophagy related genes and contributes to autophagic death [94–99]. Casticin induces ferroptosis via iron overload and increased ROS, upregulating ferroptosis markers such as HMOX1, LC3B and NCOA4. Its ability to induce ferroptosis and inhibit OS growth and metastasis has been confirmed in vivo without significant toxicity [95]. These flavonoids modulate multiple signalling pathways. Diosmetin suppresses STAT3 signalling, contributing to its antiproliferative effects and G2/M arrest [90]. Acacetin

activates JNK signalling and inhibits Wnt/β catenin, both essential to its suppression of tumor growth and metastasis. In vivo, acacetin decreases PCNA and β catenin while increasing E cadherin and p JNK, supporting its anti-metastatic potential [91, 92]. Paucatalinone A interferes with ERK1/2 signalling and inhibits proliferation partly by promoting actin depolymerization, thereby reducing also cell migration. In BALB/c mice bearing K7M2 xenografts and treated intravenously, paucatalinone A significantly reduced tumor volume and weight [93]. Hyperoside activates the TGF β pathway and promotes osteoblastic differentiation by upregulating OPN, RUNX2 and osteocalcin [96]. Hyperoside, paucatalinone A, quercetin and acacetin mainly induce G0/G1 arrest, increasing cell-cycle inhibitors and lowering cyclin D1 [91–94, 96–98]. Notably, quercetin does not significantly affect cyclin B1, suggesting additional mechanisms in G2/M regulation [97]. Several flavonoids also exhibit anti-metastatic effects: quercetin and acacetin reduce HIF 1α, VEGF and MMP-2, 7 and 9, thereby inhibiting migration and invasion. In vivo, both compounds reduced primary tumor growth and metastatic lesions with IP or oral administration [91, 98]. Lastly, Cinnamtannin suppresses metastasis mainly via the miR-1281/Peptidylprolyl Isomerase F (PPIF) axis. By reducing PPIF, a component of the mitochondrial permeability transition pore (MPTP), it promotes mitochondria mediated apoptosis and inhibits proliferation, migration and invasion. In vivo, cinnamtannin treatment decreased tumor volume and weight [100].

Naphthoquinones

Naphthoquinones, widely present in higher plants, are of great interest due to their pharmacological and anticancer properties [101]. Acetylshikonin, deoxyshikonin, plumbagin, chimaphilin and shikonin all exert strong pro-apoptotic effects, primarily via the mitochondrial pathway, as shown by ANXV/PI assays. They promote caspase cleavage, increase pro-apoptotic proteins and reduce anti-apoptotic proteins [102–107]. Acetylshikonin and deoxyshikonin activate apoptosis through the p38 MAPK pathway, increasing phosphorylation of ERK1/2, JNK1/2 and p38 [102, 103]. Plumbagin induces both mitochondrial apoptosis and ER stress, as indicated by increased GRP78 and GRP94 levels [104]. Chimaphilin exhibits additional anti-invasive and anti-migratory effects by downregulating IGF 1R expression and activation [105]. It also blocks TGF β1 induced EMT by reducing Smad2/3 phosphorylation, thereby inhibiting TGF β1 signalling and EMT progression [106]. Shikonin likewise inhibits migration, consistent with changes in EMT markers [107].

Phenolic acids

Phenolic acids, a subclass of plant phenolics, show promising cytotoxic anticancer activity by promoting apoptosis, reducing proliferation and targeting angiogenesis, differentiation and metastasis [67]. Oleuropein and grifolic acid both exhibit strong antiproliferative effects on OS cells. Oleuropein's cytotoxicity increases in a dose and time dependent manner [108]. It induces autophagy, markedly upregulating AMBRA1, ULK1 and BNiP3L, similarly to Indicaxanthin in Caco-2 cells [109]. Concurrently, p62 levels rise while LC3 expression is reduced, consistent with altered autophagic flux and likely mitophagy [110]. Grifolic acid primarily induces necrosis rather than apoptosis. ANXV/PI staining shows a predominance of necrotic cell death. It disrupts mitochondrial function, decreasing mitochondrial membrane potential (MMP) detected by JC 1 staining, leading to energy failure and cell death. In vivo, grifolic acid displays localized tumor cytotoxicity, reducing tumor growth, cell viability and lung metastases and prolonging survival, without evident systemic toxicity [111].

Phenylpropanoids

Phenylpropanoids are a major group of plant secondary metabolites produced in response to biotic and abiotic stress, including lignans and monolignols [70] Natural and synthetic phenylpropanoids have attracted attention as antioxidant and anticancer agents [112]. Angedahurin and Anticarin B both promote cell death via distinct mechanisms. Angedahurin induces apoptosis in a dose dependent manner [113]. Anticarin B impairs autophagy by blocking autophagosome–lysosome fusion and inhibits the chaperonin activity of the TRiC complex, disrupting folding of regulatory proteins such as STAT3. This leads to loss of STAT3 function, cellular stress and apoptosis. In vivo, Anticarin B significantly reduces tumor growth and improves survival [114]. Geiparvarin mainly inhibits metastasis rather than directly inducing cell death. It downregulates miR-3912-3p, thereby altering ANGPTL4 expression, a key factor in metastasis. In vivo, geiparvarin markedly reduces the size and number of lung tumor nodules without significant side effects or weight loss [115].

Physakengoses

Physakengoses are newly discovered compounds who have demonstrated promising anti-tumor effects. Physakengose G promote cell death through the mitochondrial apoptosis pathway and exerts its anti-cancer effects such as inhibition of cell proliferation and survival by altering the EGFR/mTOR signalling pathway. It also increases LC3B II levels, inhibiting autophagosome formation, indicating impaired autophagic flow due to the lysosomal dysfunction [116].

Polyketides

Polyketides are natural products from plants and bacteria that represent an important source of biologically active and clinically valuable molecules, including anticancer agents [117]. Maclurin induces apoptosis through caspase 3 independent PARP inactivation and a pro-oxidant mechanism that increases intracellular ROS. ANXV/PI staining shows enhanced early apoptosis, while TUNEL assays confirm increased DNA fragmentation. Cleaved PARP rises without changes in caspase 3, consistent with caspase 3 independent apoptosis. Maclurin also exerts anti-metastatic effects by activating p38 and inhibiting ERK [118].

Stilbenes

Stilbenes possess notable antioxidant, pro apoptotic and anti-inflammatory properties with low in vivo toxicity, making them attractive for cancer prevention and therapy [119]. Resveratrol, oxyresveratrol and glaucocalyxin A all induce apoptosis in OS cells via the mitochondrial pathway, as shown by ANXV/PI staining. Resveratrol inhibits the Akt pathway by reducing p Akt, thereby limiting cell survival and proliferation. It also exerts epigenetic effects by increasing methylation of IL 6 and IL 8 promoters (MSRE PCR), reducing secretion of these pro-inflammatory cytokines and dampening the tumor-promoting inflammatory microenvironment. Additionally, resveratrol promotes osteoblastic differentiation by upregulating Osx, which in turn enhances expression of SPP1, ALPL, COL1A1 and BGLAP [67]. Oxyresveratrol and glaucocalyxin A both target the JAK/STAT3 pathway, decreasing phospho STAT and JAK2 phosphorylation, thereby inhibiting STAT3 activation [120, 121]. Glaucocalyxin A further induces oxidative stress, increasing ROS and shifting the GSSG/GSH ratio, and causes G2/M arrest. In vivo, glaucocalyxin A suppresses tumor growth and STAT3 activation, confirming its mechanism of action [121].

Tannins

Tannins are a heterogeneous class of natural compounds with chemo-preventive and therapeutic potential against cancer, acting on many pathways involved in tumor development [65]. Urolithin B induces late apoptosis by upregulating Bax and p53 and also causes necrosis. p53 can accumulate in mitochondria and induce necrosis by interacting with cyclophilin D, promoting mitochondrial permeability transition pore (MPTP) opening. Bax overexpression and Bcl 2 downregulation further extend MPTP opening, leading to mitochondrial swelling, loss of ATP synthesis and increased ROS, culminating in both apoptosis and necrosis. Urolithin B also inhibits migration and metastasis by downregulating MMP 2 and MMP 9 [122].

Terpenoids

Terpenoids are one of the largest classes of plant derived natural products and exhibit diverse antitumor activities, including antiproliferative, pro-apoptotic, anti-angiogenic and anti-metastatic effects [63, 123]. Those studied in osteosarcoma include celastrol, raddeanin A, hederoside C, degalactotigonin, bruceine D, bruceantinol, MO OH Nap, ginsenoside Rh2, elemene and dioscin [124–136]. Celastrol, raddeanin A, hederoside C, degalactotigonin, bruceine D, bruceantinol, ginsenoside Rh2, elemene and dioscin induce apoptosis via the intrinsic pathway, as confirmed by ANXV/PI assays [124, 126–136]. MO OH Nap, a novel tropolone, induces apoptosis by activating the unfolded protein response (UPR), increasing ATF4, IRE1 and p eIF2. It also disrupts iron homeostasis by upregulating TFR and related genes and alters purine and pyrimidine metabolism, while inhibiting migration and invasion [125]. Raddeanin A, hederoside C, bruceine D and bruceantinol all target STAT3 signalling, reducing phosphorylated form of STAT3 and then its downstream targets [127–131]. Raddeanin A and hederoside C also modulate JNK: raddeanin A enhances JNK and c JNK phosphorylation and activates JNK/c Jun, whereas hederoside C suppresses JNK activity. In vivo, both reduce tumor growth by regulating JNK/c Jun and STAT3 [127, 129]. Degalactotigonin inhibits the Hedgehog/Gli1 pathway by inactivating GSK 3β, reducing Gli1 transcription and expression and its target genes [133]. Raddeanin A and ginsenoside Rh2 inhibit NF κB signalling. Raddeanin A suppresses IκBα phosphorylation and nuclear localization of p65 [126]. Ginsenoside Rh2 diminishes NF κB activation, activates MAPK signalling, and inactivates PI3K/Akt/mTOR by reducing p PI3K, p Akt and p mTOR, amplifying apoptotic signalling [132]. Elemene downregulates the renin–angiotensin system, decreasing renin, AngII and ACE levels in a dose dependent manner [134]. Dioscin upregulates miR-16-5p, which inhibits the HuR/Pim1 pathway involved in survival, proliferation and stress responses; this mechanism, confirmed in vivo, leads to reduced tumor growth and metastasis [136]. Bruceine D and degalactotigonin also affect cell cycle progression. Bruceine D induces G0/G1 arrest in MNNG/HOS cells by downregulating key cell cycle regulators [130]. Degalactotigonin primarily causes G2/M arrest by increasing p21 and altering cyclin D1. This is linked to DNA damage, as shown by increased phospho H2A.X foci, a marker of double-strand breaks, indicating impaired DNA repair [133].

Natural compounds as adjuvant in osteosarcoma: sensibilization and chemotherapy?

In different studies, natural compounds have been shown to exert chemosensitizing effects on cancer cells, enhancing the efficacy of chemotherapeutic agents and allowing dose reduction, thereby mitigating adverse effects [69].

Alkaloids

Among alkaloids, voacamine and piperlongumine have been most studied for synergistic activity with chemotherapy [1, 2]. Voacamine is not cytotoxic when administered alone but markedly increases the sensitivity of OS cells to DOX. Its chemosensitizing action is mainly due to interaction with P-glycoprotein (P gp), a transporter that extrudes many cytotoxic drugs and sequesters DOX in acidic vesicles, lowering its nuclear concentration. By acting as a competitive P-gp antagonist, voacamine enhances intracellular and nuclear accumulation of DOX and restores its cytotoxicity. This effect is absent in SAOS 2 WT and Me30966 cells, which do not express surface P-gp, confirming target specificity. MTT assays show that pretreatment with voacamine followed by low dose DOX reduces cell survival to about 50%, and combination treatment produces significant cytotoxicity even with non toxic DOX concentrations. Thus, comparable antitumor effects can be achieved with lower DOX doses, potentially reducing hepatotoxicity and cardiotoxicity in vivo. Combined treatment with voacamine and DOX also induces evident morphological changes under phase contrast microscopy [137]. Voacamine is not cytotoxic when administered alone but markedly increases the sensitivity of OS cells to DOX. Its chemosensitizing action is mainly due to interaction with P-glycoprotein (P-gp), a transporter that extrudes many cytotoxic drugs and sequesters DOX in acidic vesicles, lowering its nuclear concentration. By acting as a competitive P-gp antagonist, voacamine enhances intracellular and nuclear accumulation of DOX and restores its cytotoxicity. This effect is absent in SAOS 2 WT and Me30966 cells, which do not express surface P-gp, confirming target specificity. MTT assays show that pretreatment with voacamine followed by low dose DOX reduces cell survival to about 50%, and combination treatment produces significant cytotoxicity even with non toxic DOX concentrations. Thus, comparable antitumor effects can be achieved with lower DOX doses, potentially reducing hepatotoxicity and cardiotoxicity in vivo. Combined treatment with voacamine and DOX also induces evident morphological changes under phase contrast microscopy [86].

Flavonoids

Quercetin is a flavonoid that enhances CDDP sensitivity in OS cells by modulating the miR-217/KRAS axis. Co treatment with quercetin and CDDP for 24 h lowers the CDDP IC50 from 6.12 μM (CDDP alone) to 4.21 μM and significantly increases apoptosis, as shown by flow cytometry. This enhanced sensitivity correlates with miR-217 upregulation and KRAS downregulation at both mRNA and protein levels. Inhibition of miR-217 with a specific antagomir reverses these effects, restoring proliferation, migration, and invasion, and confirming that quercetin acts primarily through miR-217 mediated KRAS suppression [138].

Naphthoquinones

Shikonin (SHK) has poor solubility and relatively modest anticancer activity when used alone, but its efficacy is strongly enhanced by combination with valproic acid (VPA), a histone deacetylase inhibitor (HDACI). VPA increases histone acetylation and chromatin accessibility, thereby sensitizing OS cells to shikonin. Co treatment with SHK and VPA produces more pronounced inhibition of proliferation and higher cytotoxicity than SHK alone at the same concentrations. The combination also exerts a synergistic anti migratory effect, with VPA amplifying shikonin induced reductions in mesenchymal markers such as N cadherin, Vimentin, Snail and Slug, and increasing E cadherin, consistent with impaired epithelial–mesenchymal transition. Flow cytometry demonstrates that VPA significantly augments SHK induced apoptosis. This is accompanied by decreased expression of anti apoptotic proteins, increased pro apoptotic proteins, and marked accumulation of reactive oxygen species (ROS). ROS generation is a key driver of the observed apoptosis. Co treatment upregulates EGR1, a transcription factor that links ROS accumulation to apoptosis, at both mRNA and protein levels; ROS scavenging reduces EGR1 expression, confirming ROS dependence. Elevated EGR1 correlates with Bax upregulation, Bcl 2 downregulation, and increased cleavage of caspase 3 and PARP, indicating activation of the ROS–EGR1–Bax apoptotic axis. EGR1 activation is mediated by MAPK signaling in response to ROS and directly stimulates Bax transcription by binding its promoter. Strikingly, SHK–VPA co treatment achieves an antiproliferative and pro apoptotic effect comparable to DOX in OS models. In vivo experiments in nude mice bearing subcutaneous OS xenografts and treated by intraperitoneal co administration confirm the in vitro data, showing reduced tumor growth and decreased Ki67 expression, with VPA further enhancing the antitumor effect [107].

Phenolic acids

Oleuropein (OLEU) is a phenolic compound from olive fruits and leaves of Olea europaea with demonstrated cytotoxicity against cancer cells. When combined with DOX, OLEU shows a clear synergistic effect, allowing lower DOX concentrations while maintaining or

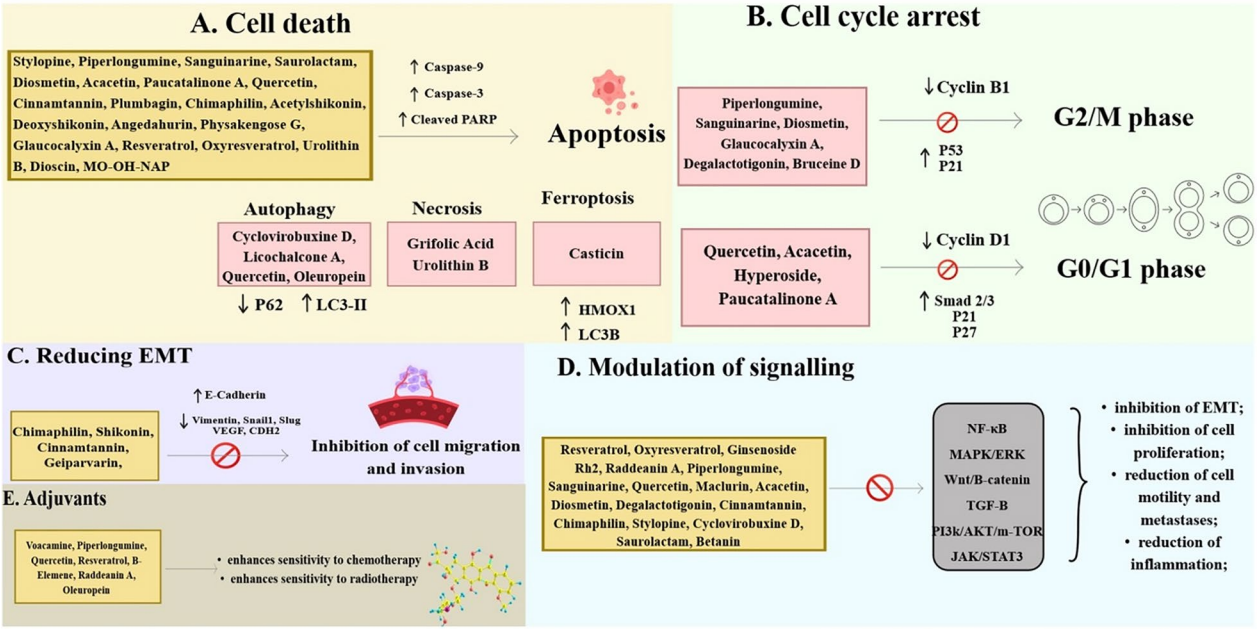

Fig. 3 Schematic draw of the main mechanisms and factors through which natural compounds exert antitumor effects. (**A**) Induction of apoptosis, ferroptosis, autophagy or necrosis, modulating specific markers and cell death pathways. (**B**) Induction of cell cycle arrest in the two checkpoints (G2/M and G0/G1), regulating key proteins such as cyclins and tumor suppressor proteins. (**C**) Inhibition of epithelial-mesenchymal transition (EMT), with the consequent inhibition of cell migration and invasion. (**D**) Modulation of oncogenic signalling pathways, such as NF-kB, PI3K/AKT/mTOR and JAK/STAT3, contributes to the reduction of cell proliferation, inflammation, metastasis and motility. (**E**) Activities of natural compounds as adjuvant to enhance the effectiveness of chemotherapy and radiotherapy

improving antitumor efficacy. MTT assays indicate that the combination significantly inhibits OS cell proliferation and induces cell cycle arrest at the G2/M phase.

Both OLEU and DOX alone increase the expression of several autophagy related genes—AMBRA1 (Activating Molecule in BECN1 Regulated Autophagy Protein 1), ULK1 (Unc 51 Like Autophagy Activating Kinase 1) and BNiP3L (BCL2 Interacting Protein 3 Like)—suggesting activation of a protective autophagic response. In contrast, co treatment neutralizes this induction and restores their expression to near control levels. Consistently, the combination reduces LC3 (Microtubule Associated Protein 1 A/1B Light Chain 3) at mRNA and protein levels and enhances p62 accumulation, indicating inhibition and blockade of autophagic flux.

Metabolomic analysis reveals that OLEU–DOX significantly depletes metabolites essential for tumor cell survival and growth, including glutamate, glutathione, phosphocreatine, ATP, uridine diphosphates (UDPs), phosphocholine and glycerol-phosphocholine. Overall, these results suggest that OLEU amplifies DOX cytotoxic effect by suppressing the autophagy mediated survival response and by targeting metabolic pathways critical for OS cell viability [110].

Stilbenes

Resveratrol (RSV) and polydatin are stilbenes investigated as potential adjuvants in OS therapy. Resveratrol, when combined with chemotherapeutic agents such as DOX and CDDP, significantly enhances their antiproliferative effects. Pretreatment with RSV reduces OS cell viability more than either RSV or the chemotherapeutic agents alone, enabling the use of lower drug concentrations while still achieving strong cytotoxicity [74]. Polydatin has been mainly evaluated as a radiosensitizer. It promotes OS cell differentiation and, when combined with ionizing radiation, drives cells toward terminal osteogenic differentiation. The osteogenic action of polydatin is evidenced by increased bone alkaline phosphatase (BAP) activity and decreased hepatic alkaline phosphatase (HAP) activity. Alizarin Red staining confirms enhanced osteoblast mediated mineralization and the formation of a more compact cell layer. In these cells, β catenin relocalizes to the plasma membrane, a pattern associated with a less invasive phenotype. Pre-treatment with polydatin for 96 h significantly increases sensitivity to low radiation doses, resulting in greater loss of viability compared with unpretreated cells. Clonogenic survival assays confirm a reduction in surviving fractions, demonstrating radio-sensitization. At the molecular level, mRNA analysis shows increased expression of osteopontin and Notch2 and decreased Notch1 levels, supporting induction of terminal differentiation and reduced tumor aggressiveness after polydatin plus radiotherapy [139].

Terpenoids

β Elemene and Raddeanin A are terpenoids studied for their ability to enhance chemotherapy efficacy [128, 135]. β Elemene displays strong synergistic effects when combined with DOX in DOX-resistant OS cells. Co treatment lowers the IC50 of DOX from 32.67 μg/mL to 7.75 μg/mL in MG63/DOX cells and from 44.16 μg/mL to 7.22 μg/mL in Saos 2/DOX cells. Combination index (CI) values of 0.42 and 0.30, respectively, confirm synergy. Flow cytometry shows increased apoptosis in the combination group, associated with reduced Bcl 2 expression and increased Bax and cleaved caspase-3. β Elemene plus DOX also modulates redox homeostasis: it markedly downregulates peroxiredoxin 1 (Prx 1), an antioxidant protein linked to chemoresistance, increases intracellular ROS and decreases glutathione (GSH). These changes likely contribute to restored DOX sensitivity. In a Saos 2/DOX xenograft mouse model, the combination suppresses tumor growth more effectively than single treatments and inhibits angiogenesis; CD31 immunohistochemistry reveals a significant reduction in microvessel density in tumor tissues [135]. Raddeanin A similarly potentiates DOX activity in resistant OS cells. Co treatment increases intracellular DOX fluorescence and reduces calcein AM efflux, indicating enhanced drug uptake and decreased efflux. Flow cytometry detects a higher percentage of apoptotic cells compared with either treatment alone. The expression of MDR1 and MRP1, major mediators of multidrug resistance, is significantly reduced following combination treatment. This effect is associated with inhibition of STAT3 signaling, as shown by decreased levels of phosphorylated STAT3 (p STAT3) and phosphorylated JAK2 (p JAK2). STAT3 overexpression elevates MDR1 and restores DOX resistance, whereas STAT3 inhibition sensitizes resistant cells, confirming that Raddeanin A acts on the STAT3–MDR1 axis. In vivo, these findings are supported by experiments in BALB/c nude mice orthotopically injected with KHOSR cells into the tibial medullary cavity and treated intraperitoneally with Raddeanin A. The combination with DOX significantly reduces tumor growth and increases apoptosis compared to single agents, demonstrating that Raddeanin A can both enhance DOX efficacy and partially reverse multidrug resistance in osteosarcoma [128].

Conclusions and prospects

OS is still a difficult cancer to treat due to its high aggressiveness, the tendency to early metastasis and often unfavourable prognosis, with survival rates showing little improvement over time. Conventional chemotherapy treatments, such as DOX and CDDP, although essential are associated with serious side effects and the development of resistance, which limits therapeutic efficacy and patients' quality of life. There is an important need to explore new therapeutic strategies that can enhance the effectiveness of existing treatments while reducing their toxicity, supporting the fight against cancer and preventing the formation of metastases [7].

Numerous in vitro and in vivo studies have demonstrated that these compounds can modulate the main signal pathways involved in osteosarcoma (see Fig. 3). According to the information collected in this paper natural compounds represent a promising opportunity for the treatment of osteosarcoma, thanks to their ability to directly target pathways critical for cancer cell survival, proliferation, metastasis and improve the effectiveness of conventional treatments [75].

The results from the studies reviewed suggest that natural compounds also can act in synergy with chemotherapy agents, enhancing their therapeutic effects while potentially reducing the side effects typically associated with high-dose chemotherapy. These compounds not only enhance drug sensitivity but also possess anti-inflammatory, antioxidant, and apoptotic properties, which contribute to their therapeutic potential [75, 76]. However, while the in vitro and in vivo evidence is promising, there are still important challenges to overcome before these natural compounds can be used into clinical practice.

One of the main limitations is that the experiments are all different, with a lack of standardized experimental protocols, which makes it difficult to compare the various compounds analyzed in the various studies. It would therefore be useful to have a more systematic approach, using shared experimental models both in terms of methods and dosages used, but above all standardizing the pathways studied in order to make the analyzes more effective and identify common therapeutic strategies. The ideal standard for the research on natural compounds in OS treatment should prioritize the study of main mechanisms that can counteract tumor progression, such as apoptosis [67, 80–82, 84, 85, 90–93, 113, 116, 118, 122, 124, 126–136]. Since mitochondrial apoptosis is frequently analysed in many studies, it would be useful to standardize this process by focusing on the main markers, such as pro-apoptotic proteins like BCL2 Associated X (Bax) and anti-apoptotic proteins like B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra-large (Bcl-xL). In addition, since angiogenesis its not consistently assessed in studies should instead be the focus of standardized analysis, due to its important role in metastasis formation [72, 73, 135]. Analysing angiogenic markers such as VEGF and VEGFR2 would be useful to comprehensively understand how natural compounds can influence tumor vascularization and metastasis formation. Also, it would be useful to focus on the analysis of core signalling pathways known to play a major role in osteosarcoma, such as JAK/STAT3 and Akt/mTOR, for example by studying

the phosphorylation levels of STAT3, Akt, and mTOR, as well as downstream effectors like c-Myc and Skp2.

On other hand, it would be useful if the adjuvant potential of the natural compounds was always tested in the studies. In this case the ideal standard would be not only to use co-treatment, as done in most studies, but to use a dual approach involving also the pre-treatments with the natural compound at least for 48 h [67, 86, 128, 135, 138]. Pre-treatment experiments could reveal whether these compounds make tumor cells more sensitive to chemotherapy, potentially overcoming mechanisms of chemoresistance. Co-treatment studies would instead evaluate the synergistic effects of combining natural compounds with chemotherapy.

It would also be important to evaluate the safety of the compounds used. Although many of them have a low toxicity index, which makes them safe for use, it would be appropriate to evaluate their long-term effects, from the perspective of the risk-benefit ratio.

In conclusion, the results highlighted that, with more systematic, standardized approaches, and subsequent clinical evaluation, natural compounds could represent a promising aid against osteosarcoma, offering new hopes for more effective and safe therapies, proving to be much more than simple adjuvants that could play a key role in future OS therapies.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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