Chapter

Phenolic Compounds – An Emerging Group of Natural Compounds against Leukaemia: *in vitro*, *in vivo* and Clinical Applications

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Abstract

Leukaemia is the most common cancer in children under 15 years of age as well as the most common blood cancer in people older than 55. The use of all *trans* retinoic acid (ATRA) in combination with arsenic trioxide (ATO) for acute promyelocytic leukaemia (APL) and tyrosine kinase inhibitors for chronic myeloid leukaemia (CML) respectively, have improved survival rates. However, new, natural therapies are constantly being sought after to overcome issues with resistance, side effects and specificity. As a result of their range of health benefits, including anticancer properties, phenolic compounds have been extensively studied over the past two decades. One on hand, *in vitro* and *in vivo* studies highlight both the inhibitory as well as differentiation inducing effects of phenolics on different leukaemia types. On the other hand, clinical trials to date have shown their beneficial effects (decrease in the absolute lymphocyte count and lymphadenopathy) in CLL (Chronic lymphoblastic leukaemia) patients. Promising therapeutic candidates for future use include epigallocatechin-3-gallate, coumarin, and gallic acid, with the latter ideally used in combination with the conventional drugs daunorubicin and cytarabine.

Keywords: Leukaemia, phenols, anticancer, differentiation therapy, apoptosis

1. Introduction

Leukaemia is a malignancy which is characterised by an uncontrolled increase in immature blood cells, termed blasts, in the bone marrow [1]. As a result, these cells permeate the bone marrow and prevent haematopoiesis from occurring normally. Such blasts eventually penetrate into the bloodstream and spread into organs [2]. The earliest observations and descriptions of cases of leukaemia were recorded by Alfred Velpeau, Alfred Donné and John Hughes Bennett [3–5]. Rudolf Virchow is credited with coining the term 'leukaemia' in 1847, from the two Greek words 'leukos' and 'helma', which mean 'white blood' [6].

Broadly, leukaemia can be classified as either acute or chronic. In acute leukaemia, the proliferating cells are very immature, while in chronic leukaemia, these cells have a more mature phenotype [7]. Furthermore, both types are subdivided into

myeloid, lymphoid and mixed lineages [8]. On one hand, in acute myeloid leukaemia (AML), these blasts are termed myeloblasts while they are lymphoblasts in acute lymphoblastic leukaemia (ALL). On the other hand, the mature cells are granulocytes or neutrophils in chronic myeloid leukaemia (CML) and are lymphocytes in chronic lymphatic leukaemia (CLL). In general, both chronic leukaemias and AML are more common in adults while ALL is generally prevalent in children [9–12].

Acute and chronic leukaemias differ in terms of onset time. In acute leukaemia, cell proliferation occurs rapidly in days, while in chronic leukaemia, the process is slower and takes months or years [13, 14]. As a result, in acute leukaemia, lack of treatment results in death within a time frame of weeks or months while in chronic leukaemia, this may be either months or years. The signs and symptoms of both types of leukaemia also vary. In acute leukaemia, the rapid proliferation of white blood cells causes bone discomfort, aches as well as swelling in the lymph nodes. The initial symptoms include anaemia, fatigue, fever and swelling in the liver and the spleen [15]. Patients with chronic leukaemia may also show similar symptoms but if anaemia is evident, it is milder than in acute leukaemia. Moreover, most patients diagnosed with chronic leukaemia do not show symptoms at the time of diagnosis [16].

In the following sections, current treatments for different leukaemia subtypes are discussed, as well as their drawbacks. Such disadvantages pave the way for the need for alternative therapies, whereby studies show that phenolic compounds are very promising candidates in this regard.

2. Leukaemia prevalence, current treatments and challenges

Leukaemia is the most common cancer in children under 15 years of age and accounts for 32% of cancers in children of this age. For patients under 20 years of age, leukaemia accounts for 25% of cancers. The most common childhood cancer, ALL, constitutes 23% of childhood cancers and between 75% to 80% of childhood leukaemia cases. AML follows ALL and encompasses between 15% to 20% of childhood leukaemia [17, 18]. Leukaemia is also the most common blood cancer in people older than 55.

Though the treatment offered to a patient diagnosed with leukaemia depends on the leukaemia type, the primary options for treatment of leukaemia remain chemotherapy and radiotherapy. Chemotherapy drugs for AML include cytarabine, daunorubicin, doxorubicin and idarubicin [19]. Where possible, a bone marrow or stem cell transplant is also used following remission. In the latter, though the procedure may result in complications, recovery rates are good [15]. For AML, the intensive chemotherapy treatment administered to the patient as an induction treatment and as consolidation treatment. In the former, the aim is to achieve remission, while the purpose of the latter is relapse prevention [20, 21]. The use of induction and consolidation therapy together with an autologous stem cell transplant results in both a high relapse risk and a high mortality, while the use of consolidation therapy together with allotransplation results in a lower relapse risk but a higher mortality due to risks associated with graft versus host [22].

Although chemotherapy is widely used to treat a variety of cancers, it is broadly cytotoxic to normal tissues. Chemotherapy needs to be administered in more than one cycle since both the proliferating and resting phase cells possess the genetic abnormality. As a result, one chemotherapy cycle alone is not enough to kill all the leukaemic cells [23]. Chemotherapy drugs are classified into five major classes based on their structure and mechanistic action. These are: alkylating agents, topoisomerase inhibitors, antitumour antibiotics, antimetabolites and microtubule inhibitors. Alkylating agents such as cisplatin act by damaging DNA and inhibiting

transcription and protein synthesis [24]. Topoisomerase inhibitors like etoposide inhibit DNA topoisomerase from releasing supercoils during DNA replication [25]. Standard chemotherapy drugs such as daunorubicin and doxorubicin fall under the class of antitumour antibiotics which inhibit enzymes involved in DNA replication [26], while cytarabine is an antimetabolite which disrupts the S phase of the cell cycle [27]. Finally, microtubule inhibitors such as paclitaxel interfere with the M phase of the cell cycle, which results in the inhibition of mitosis [28].

For AML patients younger than sixty years of age, chemotherapy results in remission rates of between 50% to 75%, with most suffering a relapse. The incidence in AML is bimodal, with remission rates being lower for older patients and relapse rates being higher [21]. This relapse is a result of haematopoietic stem cells which survive the chemotherapeutic drug treatment and regenerate. Currently five year survival rates are estimated to be around 30% for AML [29, 30]. Moreover, standard chemotherapy for AML may result in side effects including myelosuppression, tumour lysis syndrome and hepatotoxicity [31].

While chemotherapy remains the standard treatment for AML, the use of other drugs has greatly improved survival rates for about 30% of AML cases. Such patients present with FLT3 mutations, with FLT3 being a tyrosine kinase vital for the differentiation of progenitor cells into both myeloid and lymphoid lineages. The first drug approved as an FLT3 inhibitor was Midostaurin, which since 2017 has been used a treatment for FLT3 mutant AML in combination with standard chemotherapy [32, 33]. In 2018, the second FLT3 inhibitor Gliteritinib was approved as a treatment for patients who were found to be resistant to other treatments [34]. Patients with FLT3 mutations are likely to relapse as elimination of cells harbouring the FLT3 mutation is very problematic. Moreover, some patients also become resistant to FLT3 inhibitors after treatment [35].

In AML subtype APL, treatment involves the use of all *trans* retinoic acid (ATRA) and arsenic trioxide (ATO) as induction therapy, combined with mild chemotherapy. This treatment, termed differentiation therapy, has converted the prognosis of APL from poor to favourable. Through differentiation therapy, blasts differentiate, resulting in a decline in proliferative capacity, followed by apoptosis or terminal differentiation initiation. This method contrasts highly with chemotherapy which is generally nonspecific and is often accompanied by highly toxic side effects [36]. Moreover, it is also advantageous in that while it causes terminal differentiation, it does not result in bone marrow hypoplasia, and unlike chemotherapy, the proliferating cells are not killed but their maturity is induced, leading to death [37–40].

More than 98% of APL patients possess the characteristic translocation t(15;17), which results in the fusion between two genes - the PML gene and the RARα. As a result, the fusion protein PML-RARα is formed. PML-RARα is conformationally changed by ATRA at concentrations between 10^{-7} and 10^{-6} M, resulting in co-repressor dissociation and co-activator activation, leading to a relaxation in chromatin, the activation of transcription of genes involved in differentiation, resulting in the terminal differentiation of promyelocytes to granulocytes [41, 42].

Three decades ago, APL was fatal as a result of coagulation disorders, and via anthracycline based chemotherapy, the prognosis was still poor for approximately 70% of patients. Differentiation therapy using ATRA and ATO has resulted in complete remission (CR) for around 85% of patients, and 70% of patients being cured. The use of ATRA as a differentiating agent to differentiate promyelocytes into granulocytes was first discovered by Breitman *et al* in 1980. A problem that has been encountered with the use of ATRA is ATRA resistance. This has improved through the use of ATO combined with ATRA, yet drug resistance to ATRA and ATO remains an issue [43].

Moreover, though ATRA has been pivotal in the treatment of APL, this treatment may result in another complication known as retinoic acid syndrome or

differentiation syndrome (DS). It has been found to occur in around 2% to 27% of children with APL who are treated with ATRA, and in up to 50% of patients. This may result in pulmonary haemorrhage, renal failure, as well as heart failure and for this reason is termed life threatening. Differentiation syndrome typically occurs around a week or two following the start of ATRA and/or ATO therapy [44]. If DS is severe and has resulted in pulmonary or renal dysfunction, the use of ATRA is ceased [45–48]. Compared to patients who do not develop this complication, patients with DS have a lower overall free survival and event free survival [49]. Though the exact mechanism of DS is not fully known, the main key player is thought to be an excessive inflammatory response. This response stems from leukaemic cells during their differentiation process, and is due to a higher level of chemokine production and adhesion molecules on APL cells. Inflammation leads to capillary leak syndrome and blast cells infiltrating organs such as the lungs, and organ failure [50]. Treatment for DS is required early in the diagnosis, and the corticosteroid dexamethasone is administered intravenously. Corticosteroids decrease chemokine production and stop lung infiltration [51].

Reported benefits of other agents of differentiation include histone deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibitors. A key DNMT inhibitor is 5-aza-cytidine while examples of HDAC inhibitors include sodium butyrate and valproic acid [52]. Moreover, for HDAC inhibitors, the combination of both valproic acid and ATRA has been found to be beneficial for older patients with AML [53, 54]. On one hand, HDAC inhibitors act by remodeling chromatin by subduing the activity of HDACs leading to histone acetylation. This results in the expression of genes involved in the processes of differentiation as well as apoptosis. On the other hand, the effect of DNMT inhibitors is DNA hypomethylation, which leads to the re-activation of tumour-suppressor genes silenced by methylation. The use of such inhibitors stems from the fact that the differentiation block of leukaemic cells may be a result of epigenetic changes including histone acetylation and DNA hypermethylation, which may be reversed through the action of these inhibitors [55].

In contrast to other leukaemias, in CML, the genetic abnormality, termed the Philadelphia chromosome is a result of the bcr-abl protein, which was identified by Nowell and Hungerford in 1960 [56]. This oncogenic protein leads to an upregulation of tyrosine kinase and inactivation of phosphoinositide-3-kinase resulting in the proliferation of myelocytes. Imatinib is a tyrosine kinase inhibitor which acts by binding to the bcr-abl protein. This inhibition allows the cells to differentiate into mature granulocytes and subsequently die by apoptosis [57–59]. Following imatinib administration, the cytogenic response to the treatment can be at one of three levels – cytogenic response, major cytogenic response and complete cytogenic response. In 80% of the patients, it is the latter that results, and following imatinib administration, most remain stable. However in some patients, mutations in the bcr-abl tyrosine kinase domain result in lack of inhibition by Imatinib. This leads patients to rely on chemotherapy and stem cell transplantation [60, 61]. A number of unfavorable effects following Imatinib treatment have been reported and include episodic bone pain, fluid retention, lethargy and weight gain. These usually occur within the first two years of treatment, and through continued treatment, they may also be reversed [62].

For ALL, 80% of cases occur in children, and like AML, its distribution is bimodal. Though the outcomes for children have greatly improved, the same cannot be said for elderly patients, with remission rates lying between 30 and 40% for this age group [63, 64]. Treatment involves the use of chemotherapy as induction treatment, consolidation therapy and also maintenance. For the former, an anthracycline, vincristine as well as corticosteroids [65] or the Hyper-CVAD chemotherapy regimen are used [66]. For ALL patients who are Ph-positive, survival rates have improved through the use of second generation tyrosine kinase inhibitors coupled

to Hyper-CVAD [67]. Recently, great advancements have been made for relapsed or refractory ALL patients through CAR-T cell therapy [68]. Between 70-90% of these patients respond well to this treatment, however it is associated with challenges such as antigen escape, toxicity and tumour infiltration [69].

Contrastingly, many patients with CLL have indolent disease and are asymptomatic. For patients with active CLL, treatment involves the use of chemoimmunotherapy such as a combination of fludarabine, cyclophosphamide and rituximab (FCR) or bendamustine and rituximab (BR) [70, 71]. For patients with high risk ALL, other targeted treatment agents include venetoclax, ibutrinib and idelasilib [72–74]. Though toxicity and resistance remain challenges, these may potentially be alleviated by combination therapy.

3. Methods

This chapter discusses studies that have been published to date, that assess the anti-leukaemic effect of phenolic compounds. These studies are grouped into the following three categories: *in vitro*, *in vivo* and clinical trials, as outlined in the following flow chart (**Figure 1**). Both *in vitro* and *in vivo* fall under the term 'preclinical trials', which are vital prior to moving to clinical trials and aim to determine the usefulness of a drug as therapy, as well as whether treatment is accompanied by any toxicity effect. Coming from the Latin "in glass", *in vitro* refers to experimental work carried out in a laboratory, as opposed to "within the living" for *in vivo*, where experimental work is performed using living organisms. With regards leukaemia, *in vitro* studies include experimental work performed using leukaemia cell lines while *in vivo* studies utilize animal models such as mice injected with leukaemic cells. Lastly, clinical trials are performed using human subjects, and are used to confirm *in vitro* and *in vivo* results, as well as determine drug efficacy and safety, amongst other parameters.

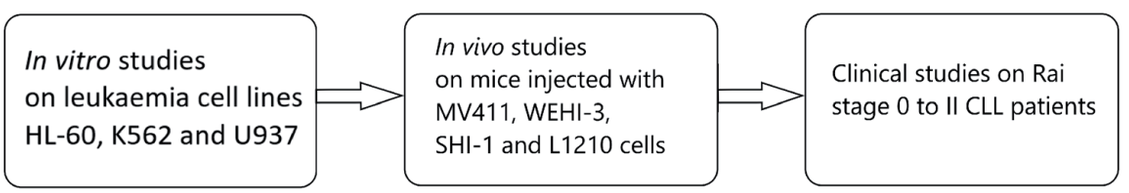

Figure 1.
A flow chart outlining the different types of studies recording the effects of phenolics on leukaemia.

4. Phenolic compounds: chemicals with a wide spectrum of bioactivity

Due to the challenges posed by the current treatments, therapies that may improve patient survival are needed. Novel treatments that are more specific and generally less toxic than conventional chemotherapy, are highly in demand. Due to their health benefits, the interest in natural products, specifically phenolic compounds, has greatly increased, making phenolics the subject of a number of research efforts over the past decade. Even more so, toxicity studies have shown that phenolics are safe and less toxic than a number of other synthetic and semi-synthetic compounds [75].

In plants, phenolic compounds are secondary metabolites consisting of an aromatic ring with one or more hydroxyl groups, which are involved in defending the plant against stress caused by drought, low or high temperatures, pathogens, restricted soil fertility and ultraviolet radiation [76, 77]. There is a wide range of such compounds and to date around 8000 of them have been identified and grouped into the following classes: phenolic acids (hydroxycinnamic and

hydroxybenzoic acids), lignans, stilbenes, coumarins, xanthones and flavonoids [78–80]. Examples of each class of phenolic compounds that have been tested on leukaemia are presented in **Table 1**.

Such phenolics are distributed to varying degrees in particular parts of plants. Caffeic acid, a major phenolic acid is widely present in fruits, tannins are high in fruit pods, wood as well as bark, and flowers are rich in flavonoids [105, 106]. These compounds have been used by man for many years in the field of traditional medicine [76]. Several studies have been carried out which demonstrate the beneficial health effects of phenols. These compounds have been found to inhibit the oxidation of low density lipoprotein (LDL) *in vivo* where LDL oxidation is associated with the formation of atherosclerotic plaques, which play a role in coronary heart disease [107]. Even more so, the phenolic compound hydroxytyrosol has been found to decrease the risk of atherosclerosis and coronary heart disease [108].

Phenolic compounds such as hydroxytyrosol, hydroxytyrosol acetate and oleuropein have also been found to hinder platelet aggregation, in so doing, decreasing the synthesis of eicosanoids such as thromboxane and thus preventing thrombosis [109, 110]. Another antiatherogenic property of phenols is their ability to reduce endothelial activation by decreasing the mRNA levels of vascular adhesion molecule-1, hence resulting in a decline in its expression. Due to this, adhesion of monocytes to endothelial cells decreases, hence preventing endothelial malfunction [111].

The antioxidant capacity of phenolic compounds has also been widely investigated. Antioxidants are vital in protecting the plant from oxidative stress [112]. Compounds possessing an *ortho*-diphenolic structure are known to display antioxidant behaviour. Examples of such compounds include the phenols hydroxytyrosol and oleuropein, whose scavenging capabilities were compared to those of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [113].

Additionally to antioxidant behaviour, hydroxytyrosol and oleuropein have been found to possess antimicrobial activity against a variety of American type culture

Class	Examples	Study	Mode of action	References
Flavonoids	Quercetin	*In vitro*	G_2M arrest	[81–83]
	Curcumin	*In vivo*	Tumour inhibition	[84–86]
	Epigallocatechin-3-gallate	Phases I and II	Decline in absolute lymphocyte count	[87, 88]
Phenolic acids				
- Hydroxybenzoic acids	Gallic acid	*In vitro* *In vivo*	G_0/G_1 arrest Tumour inhibition	[89, 90] [91, 92]
- Hydroxycinnamic acids	Cinnamic acid	*In vitro*	G_0/G_1 arrest and differentiation	[93]
Xanthones	α-mangostin	*In vitro*	Apoptosis	[94, 95]
Stilbenes	Resveratrol	*In vitro*	Apoptosis	[96]
Lignans	Syringaresinol	*In vitro*	G_0/G_1 arrest	[97]
Tannins	Tannic acid	*In vitro*	Apoptosis	[98]
Coumarins	Coumarin	*In vitro*	Apoptosis	[99–101]
Phenolic alcohols	Hydroxytyrosol	*In vitro*	Apoptosis and differentiation	[102]
Secoiridoids	Oleuropein	*In vitro*	Differentiation	[103, 104]

Table 1.
The major classes of phenolics and respective examples found to have an effect on leukaemia.

collection (ATCC) bacterial strains and clinical bacterial strains [114]. Moreover, such compounds are also anti-inflammatory agents. This is because they have been found to reduce both the release of arachidonic acid as well as production of arachidonic acid metabolites which play central roles in inflammation [115]. Also crucial to inflammation are the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). It has been reported that such enzymes are inhibited by the phenolic compound oleocanthal, in a mechanism like that of ibupforen [116].

5. Phenolic compounds and leukaemia: *in vitro* studies

Various *in vitro* studies indicate that phenols possess anticancer properties [117–120]. Phenols are commonly found in foods including nuts, fruits, vegetables and oil. Studies have shown that diets rich in phenols help prevent a variety of cancers [121–124].

Structure-activity-relationship studies have shown that the anticancer properties of these compounds vary as a result of the functional groups present in the structure, where both the hydroxylic groups present as well as the aromatic ring play an important role. With regards to hydroxylic groups, the more the number of such groups, the higher the anticancer properties. Moreover, the presence of a side chain consisting of an unsaturated fatty acid makes the phenolic compound more effective (**Figure 2**) [125, 126].

In general, phenols act by inhibiting the cell cycle, leading to apoptosis (**Figure 3**) [127–129]. In addition, phenols appear to subdue the expression of chemokines as well as cytokines and angiogenesis is stopped. Both of these are vital for tumour development regulation [130–132].

Though a number of *in vitro* studies have focused on the effect of phenols on carcinomas, gliomas, melanomas, lung cancer and breast cancer, other studies have reported the inhibitory effects of phenolic compounds on leukaemia cell lines, with most studies focusing on HL-60, U937 and K562 cells.

The HL-60 cell line was isolated in 1977 and is classified as acute myeloblastic leukaemia with maturation (M2 category in the French-American-British classification) [133, 134]. In this suspension culture, a vast majority of the cells are promyelocytes which can be induced to differentiate into monocytes or granulocytes respectively by a number of compounds such as Phorbol 12-myristate 13-acetate (PMA), sodium butyrate, dimethyl sulfoxide (DMSO) as well as all-*trans* retinoic acid (ATRA) [135]. The U937 cell line was isolated in 1974 from a patient with histiocytic lymphoma and is classified under the M4 category in the

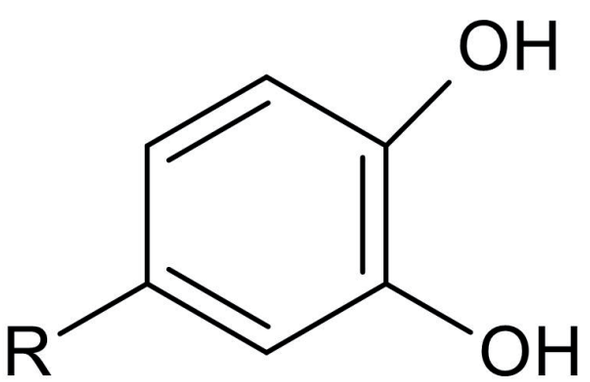

Figure 2.
The aromatic ring, the number and position of OH groups, and the presence of the unsaturated fatty acid side chain (R) influence activity.

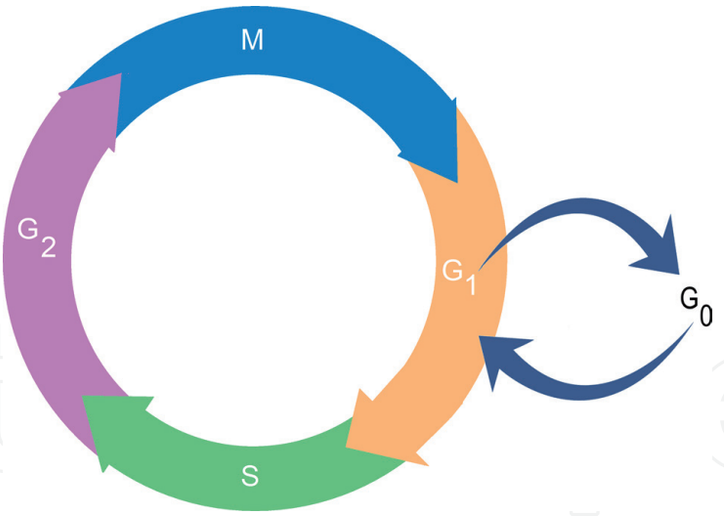

Figure 3.
The cell cycle – a process inhibited by phenolics. G_1 = Gap 1, S = Synthesis phase, G_2 = Gap 2, M = Mitosis, G_0 = resting phase.

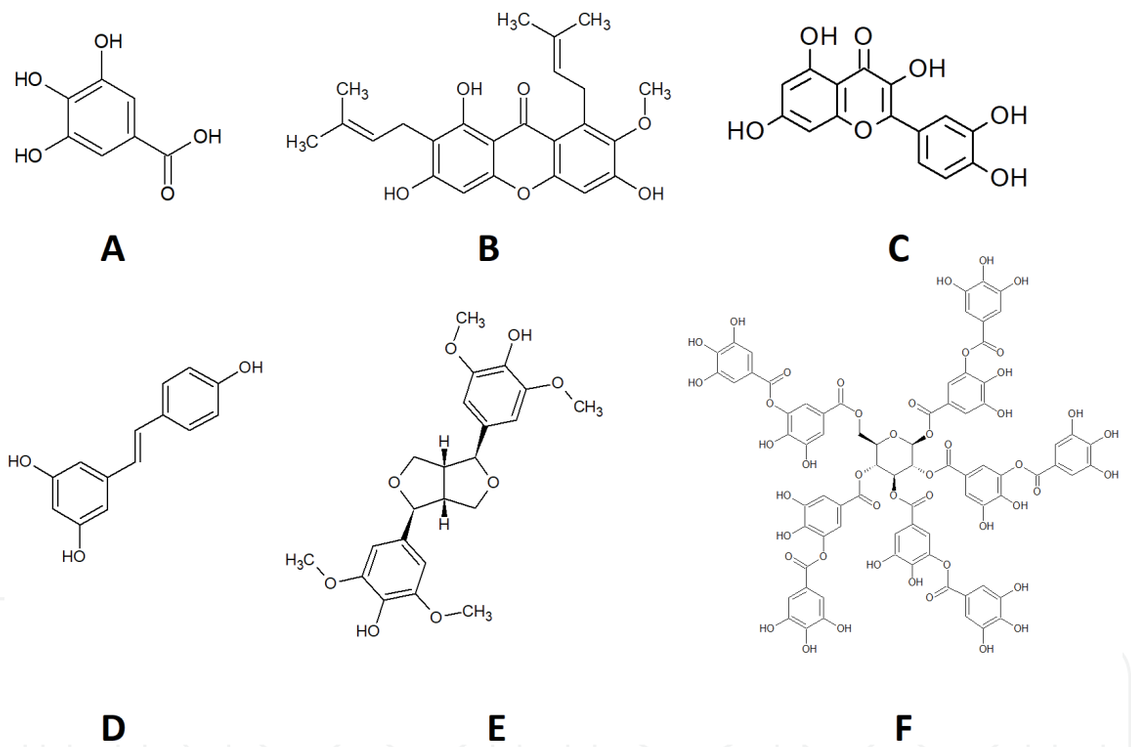

Figure 4.
Some phenolics found to possess anti-leukaemic activity. A = Gallic acid, B = Mangostin, C = Quercetin, D = Resveratrol, E = Syringaresinol and F = Tannic acid.

French-American-British classification. The cells are promonocytes and can be driven towards monocytic differentiation by PMA. For this reason, the cell line is used as a model for both monocyte and macrophage differentiation [136]. K562 is an example of erythroleukaemia [137]. It was isolated from a patient diagnosed with CML "in blast crisis", which is the final phase of the disease [138, 139]. The K562 cell line is positive for the Philadelphia chromosome, which is present in the vast majority of patients (>95%) diagnosed with CML. The cell line is termed to be proerythroblastic and studies have shown that it can be induced to monocytic, megakaryocytic and erythroid differentiation using chemicals such as proanthocyanidins for the former lineage, PMA for both the megakaryocytic and monocytic

lineage and 5-azacytidine, butyric acid and hemin for the latter lineage [140, 141]. The *in vitro* effects of phenolics on the above-mentioned cell lines will be discussed below, and the structures of some of these phenolics are shown in **Figure 4**.

5.1 Flavonoids

A number of phenolic compounds belonging to the flavonoid class have been found to have an effect on leukaemia cell lines. Quercetin is a flavonol that has been reported to inhibit the proliferation of HL-60 cells and induce their apoptosis by the activation of caspase-3, the downregulation of Bcl-2 protein and the upregulation of the Bax protein. Its effects have been found to be both dose and time dependent. The action of quercetin on the mitochondrial pathway of apoptosis also involves the inhibition of COX-2 [81]. It has also been suggested that its antiproliferative effect may be due to its capacity to inhibit both cytosolic protein kinase C as well as tyrosine protein kinase [82]. Quercetin has been found to arrest the cell cycle of both HL-60 cells and U937 cells, with treatment resulting in an increase in the number of cells in G_2M phase. For U937 cells, this effect was coupled to a decrease in cyclins D, E, E2F1 and E2F2 [83]. Furthermore, the treatment of K562 cells with quercetin results in a number of morphological changes which include nuclear fragmentation as well as nuclear chromatin condensation. It has been found to inhibit the synthesis of heat shock protein 70, which is known to be involved in regulating the processes of both cell proliferation and differentiation [142, 143].

Within the same class of flavonols, both galangin and kaempferol have been found to inhibit the growth of HL-60 cells in a dose dependent manner. For kaempferol, this was attributed to both apoptotic and non-apoptotic effects but for galangin, the increased level of caspase-3 is suggestive of apoptosis [144]. These effects were also observed for two major flavones apigenin and luteolin. For the former, treatment resulted in an increase in both caspase-3 and caspase-9 proteases as well as cytochrome c [145–147]. Furthermore, the treatment of U937 cells with apigenin resulted in the cleavage of Poly (ADP-ribose) polymerase (PARP) as well as in the activation of caspase-3, caspase-7 and caspase-9. As for quercetin, down-regulation of Bcl-2 also occurs [148].

It has been shown that, similarly to quercetin, the flavone chrysin induces both U937 cell proliferation decline and DNA fragmentation. Its apoptotic effect on this cell line has been found to involve activation of caspase-3 as well as the inactivation of Akt (protein kinase B) [149, 150]. A methylated form of chrysin, termed 5,7-dimethoxyflavone was found to inhibit the growth of YCUB leukaemia cell lines in a dose and time dependent manner. Though this effect was seen on both YCUB-2 and YCUB-5 cells, for the former, an accumulation of reactive oxygen species was observed, but this was absent in the latter, suggesting a potentially different mechanism of action. Moreover, when 5,7-dimethoxyflavone was tested in combination with anticancer drugs such as cytarabine, an antagonistic effect was observed, suggesting the use of the compound as a single agent [151].

As a flavanol, epigallocatechin-3-gallate (EGCG) has been found to induce apoptosis in both acute and chronic myeloid leukaemia. For the former, a decline in death associated protein kinase 2 is observed, and an increase in neutrophil differentiation results on treatment of acute promyelocytic leukaemia with both ATRA and EGCG [152]. For the latter, the use of both EGCG and ponatinib results in a synergistic apoptotic effect which involves the downregulation of the CyclinD1 gene and the upregulation of TGF-β2 gene [153]. It has been reported that epigallocatechin-3-gallate causes the downregulation of the 67LR gene, and the induction of apoptosis is selective to cancer cells [154].

The anthocyanin delphinidin-3-sambubioside induces apoptosis in HL-60 cells through activation of three caspases which are caspase-3, caspase-8 and caspase-9, and causes DNA fragmentation [155].

Finally, the flavonoid curcumin and the metabolite tetrahydrocurcumin have both been found to induce apoptosis and autophagy respectively both in HL-60 cells as well as in HL-60 cells resistant to cytarabine [156]. This finding has very promising applications to overcome the issues with drug resistance. Furthermore, the combination of two flavonoids curcumin and quercetin induces mitochondrial apoptosis in CML. Since used in combination, any toxic effects on normal cells are unlikely since the treatment dose is lowered [157].

5.2 Phenolic acids and their derivatives

For hydroxybenzoic acids, gallic acid has been found to possess cytotoxic activity on HL-60 cells. Furthermore, gallic acid inhibits ribonucleotide reductase and arrests the cell cycle at the G_0/G_1 phase [89, 90]. The apoptosis of HL-60 cells by derivatives of gallic acid has also been investigated, and it has been concluded that apoptosis is greater in the presence of a long hydrophobic chain [158]. One of the derivatives of gallic acid, ellagic acid has been found to accumulate HL-60 cells in the S phase as well as induce their apoptosis with an increase in caspase-3 expression and PARP cleavage. Moreover, ellagic acid also enhances the differentiation effect of ATRA on HL-60 cells, and thus may be useful in overcoming ATRA resistance [159]. Ellagic acid has also been found to induce apoptosis in B-lymphocytes obtained from untreated CLL patients. This apoptotic effect involved the formation of reactive oxygen species, activation of caspase-3 and release of cytochrome c. Interestingly, this effect was selective to cancerous B-lymphocytes, and no toxic effect was seen for B-lymphocytes obtained from healthy donors [160].

With respect to hydroxycinnamic acids, caffeic acid phenethyl ester (CAPE) and cinnamic acid were found to induce apoptosis in HL-60 cells and K562 cells respectively, where for CAPE, protein, DNA and RNA synthesis in HL-60 cells were found to be inhibited [93, 161]. CAPE treatment resulted in the stimulation of Bax, downregulation of Bcl-2 as well as activation of caspase-3, signifying an apoptotic mechanism [162]. Apoptosis of U937 cells following CAPE treatment has also been recorded, with this effect being accompanied by an increase in cytochrome c [163]. For the cinnamic acid, a dose dependent arrest in the G_0/G_1 phase has been observed. Cinnamic acid has also been found to induce differentiation in K562 cells [93].

5.3 Xanthones and stilbenes

For xanthones, the effect of α-mangostin on HL-60 cells was investigated and its apoptotic effect was found to be caspase-3 dependent [94]. Apart from α-mangostin, β-mangostin also inhibits the growth of HL-60 cells, arrests them at the G_0/G_1 phase and induces intrinsic apoptosis through the activation of caspases-3, 7 and 9 and Bax, as well as the down-regulation of Bcl-2. Like quercetin, β-mangostin inhibits heat shock protein 70 [95].

With respect to stilbenes, studies have mainly focused on resveratrol, which has been found to be a differentiation inducing agent, as well as an inducer of apoptosis. This has been observed on NB4 cells, which are a type of APL. Like the xanthone α-mangostin, treatment with resveratrol results in an increase in caspase-3 activity. Nonetheless, for both α-mangostin and resveratrol, treatment on HL-60 and NB4 cells respectively does not have an effect on the Bcl-2 protein levels. Hence this is suggestive of an alternative apoptosis pathway. Differentiation of NB4 cells with resveratrol is completely effective when the

cells are treated with both ATRA and resveratrol [96]. Furthermore, synthesized resveratrol analogues also arrest the cell cycle of HL-60 cells but do so at all three phases, G_0/G_1, S and G_2/M, contrasting with resveratrol which has been found to be phase specific [164]. It is relevant to highlight that though resveratrol is effective, it is limited by its poor bioavailability [165, 166]. Another two stilbenes namely piceatannol and sophorastilbene A both possess dose dependent cytotoxic activity on HL-60 cells with caspases 3, 8 and 9 being activated, with no changes in Bcl-2 protein expression being recorded [167].

5.4 Lignans

Within the class of lignans, (-)syringaresinol possesses anti-leukaemic behaviour. This is because it induces G_0/G_1 HL-60 cell cycle arrest in a manner that is both dose and time dependent. This is accompanied by the activation of both caspase-3 and caspase-9, DNA fragmentation and the release of cytochrome c [97].

5.5 Tannins

Tannins such as woodfordin C, cuphiin D1, cuphiin D2 and oenothein B have been found to possess cytotoxic behaviour on HL-60 cells [145, 168]. Tannic acid also induces apoptosis in HL-60 in both a time and dose dependent manner. The apoptotic mechanism was noted to involve the activation of caspases, PARP cleavage and cytochrome c release. Interestingly, tannic acid enhanced the cytotoxic effect of arsenic trioxide on HL-60 cells. This finding suggests the potential use of tannic acid in combination with arsenic trioxide [98].

5.6 Coumarins

Apoptotic activity on HL-60 cells was also recorded following treatment with 4-substituted coumarins, as well as furanone-coumarins, with an enhanced activity of caspases -3 and 9 also being recorded [99, 100]. Moreover, interestingly, coumarin was also found to induce cell death in drug resistant HL-60 cells when combined with doxorubicin [101]. This combination has great potential in overcoming the issue of drug resistance.

5.7 Phenolic alcohols and secoiridoids

While most of the effects reported referred to the inhibitory effect of phenolics, some studies have focused on their differentiating activity. Such studies have focused mainly on HL-60 cells, while other cell lines have been overlooked. Polyphenols from pomegranates and green tea, proanthocyanidins from barley and ellagic acid from fruits such as blackberries, pomegranates and strawberries have been found to induce differentiation HL-60 differentiation [159, 169–171]. Another three studies have focused on phenols from olive oil and the use of an olive leaf extract [102–104]. Two of these studies further confirm that phenolic compounds are capable of inhibiting cell proliferation and inducing differentiation in HL-60 cells. For the olive leaf extract study, the differentiation inducing compounds were found to be oleuropein and apigenin 7-glucoside [103]. The results from the study using olive oil on HL-60 cells show that dialdehydic compounds of elenoic acid with tyrosol and hydroxytyrosol are capable of inducing apoptosis and differentiation. It was reported that the effect of these two compounds was only a minor percentage of the total effect seen using the crude phenol extract [102]. Results from another study using an olive leaf extract with oleuropein as the major

constituent show that the extract is capable of inducing both apoptosis as well as differentiation in K562 cells, along the monocyte/macrophage lineage [104].

The apoptotic effects recorded for phenolic compounds on leukaemia cell lines are potentially more similar to those of antitumour antibiotics as opposed to microtubule inhibitors and alkylating agents. This is beneficial as the latter two categories are highly unspecific as they target cells by mitotic spindle inhibition or DNA adduct formation respectively.

6. Phenolic compounds and leukaemia: *In vivo* studies

In addition to the *in vitro* effects of phenolic compounds, some *in vivo* studies have also been conducted. These studies focus on the use of gallic acid, curcumin, and resveratrol, and will be discussed in this section.

Using AML xenograft tumour NOD/SCID mice models injected with MV411 leukaemia cells, the effect of gallic acid in combination with daunorubicin and cytarabine was investigated. The results show that when gallic acid was used in combination with such drugs, tumour inhibition was observed when compared to the use of the drugs alone as single agents [91].

Interestingly, both gallic acid and curcumin were found to inhibit WEHI-3 leukaemia cells *in vivo*. Using BALB/c mice injected with WEHI-3 cells, both gallic acid and curcumin caused a reduction in the weights of the livers and spleens of such mice. For gallic acid, it has been postulated that this effect occurs through the increase in macrophage phagocytosis. This finding is particularly interesting in that it contrasts highly with the enlarged spleen associated with WEHI-3 leukaemia. Moreover, both phenolics caused a reduction in the Mac-3 marker (macrophage precursor) percentage [84, 92].

For curcumin, an inhibition of CML was recorded using CML xenograft SCID mice and mice treated with curcumin had smaller tumours. Moreover, plasma exosomes of treated mice were found to contain higher levels of miR-21 [85]. Curcumin also inhibits the growth of SHI-1 leukaemia cells in SHI-1 injected SCID mice. The mechanism involves signaling of NF-kB and ERK pathways, and an activation of JNK and p38 [86].

Using mice treated with L1210 cells, resveratrol was found to increase the life span of such mice, as well as the activity of NK cells, which is an important mechanism for eradication of a tumour. Furthermore, lymphocyte proliferation and the humoral immune response were found to be enhanced following resveratrol treatment [172].

7. Phenolic compounds and leukaemia: clinical trials

In addition to *in vitro* and *in vivo* studies, the beneficial effects of phenolic compounds have also been made evident through clinical trials. The main sources of phenolics investigated in such studies have been olive oil, pomegranate juice, *Curcuma longa* and green tea. A daily short-term consumption of olive oil has been found to affect a number of biomarkers related to oxidative stress, with an increase in high density lipoprotein cholesterol and a decrease plasma oxidized low density lipoprotein being observed dose-dependently according to the phenolic content of olive oil [173, 174]. For cancer, clinical trials have shown phenolics to be effective against prostate and colorectal cancer [175, 176].

For leukaemia, the clinical trials that have been performed to date have focused on CLL and utilized olive oil and a green tea extract as the polyphenolic sources. For

one study, an olive oil rich in oleocanthal and oleacin at concentrations of 416 mg/kg and 284 mg/kg respectively, was selected. For this trial, performed in 2019, a cohort of 21 patients with CLL Rai stage 0 to II were chosen, who were not receiving any treatment. The effect of daily ingestion of 40 mL of olive oil per day for a period of six months was tested through the analysis of a number of molecular, haematological and biochemical markers at different time points. Such tests included liver function, kidney function, glucose profile, lipidemic profile and an analysis of apoptotic markers CCK18, Apo1-Fas and anti-apoptotic protein survivin. The glucose and lipidemic profiles of such patients were found to improve, the levels of the apoptotic markers CCK18 and Apo1-Fas increased and survivin decreased [177].

Similarly, on Rai stage 0 to II CLL patients, phase I (33 patients) and phase II (42 patients) clinical trials were conducted using a green tea extract (Polyphenon E), containing a standardized dose of EGCG. The results from the phase I clinical trial showed a good toleration of the extract in patients at doses ranging from 400 mg to 2000 mg twice daily, as well as a decline in both the absolute lymphocyte count as well as in lymphadenopathy. The same positive results were obtained in the phase II clinical trial, this time with a twice daily dose of 2000 mg. The side effects were reported to include nausea, transaminitis, and abdominal pain [87, 88].

8. Future perspective

The anti-leukaemic potential of phenolic compounds has been well documented through both *in vivo* and *in vitro* studies. While a number of clinical trials also show the promise of such compounds as treatments for a variety of cancers, more clinical trials on leukaemia are needed in order to ensure that the findings from *in vitro* and *in vivo* studies are confirmed, as well as determine the safety and efficacy of such treatments.

9. Conclusions

The studies presented in this chapter show the benefits of phenolic compounds, both as anti-proliferative agents as well as differentiation agents for leukaemia. These compounds have been found to arrest the cell cycle of leukaemia cells, as well as to induce apoptosis and differentiation. In a number of phenolics, such effects were noted to be selective, in contrast to chemotherapy. The promising results offer a potential alternative to the current standard treatments, in the hope that being natural products, are less toxic and are accompanied by less adverse effects. Furthermore, some phenolics show great therapeutic potential in multi-drug resistance leukaemia patients.

Conflict of interest

The authors declare no conflict of interest.

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