Review article

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INDUCTION OF APOPTOSIS IN CANCER CELLS BY PLANTS IN THE GENUS *EUPHORBIA*

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ABSTRACT

Euphorbia is an evolutionarily distinct family of plants which have long been used in traditional medicine of diverse cultures to treat various ailments including cancer. In line with their traditional applications, research over the years has supported the anticancer properties of plants in the genus *Euphorbia*, particularly through their pro-apoptotic activities against various types of cancer cell lines. The aim of this review is to provide a summary of knowledge accumulated over the past few decades regarding the pro-apoptotic activities of the myriad euphorbia species, the chemical structure of isolated compounds and emerging pro-apoptotic mechanisms, as well as discussions relating to the future prospects for research and development of potential anticancer leads from plants in the genus *Euphorbia.*

Keywords: antiproliferative, cytotoxic, apoptosis, *Euphorbia*, cancer

Introduction

Cancers are complex diseases emerging from a deregulation of the proliferation and homeostatic circuits of the cells. Decades of intense research into the molecular biology of cancers have resulted in a foundation of knowledge regarding the frequently deregulated pathways in cancers. Although far from complete, this foundation has led to the development of novel therapies for cancer treatment, both clinically approved and currently in clinical trials. However, despite these advancements, cancers still pose a major threat to public health and remain a leading cause of death globally. Cancer related mortality and morbidity is largely attributable to its extensive proliferation and invasiveness, which disrupt normal functions of affected organs. To date, pharmacotherapy has still been considered as one of the treatment options for various types of cancers. However, certain anticancer drugs are associated with serious side-effects, compromising the quality of life of cancer patients. Moreover, cancers may develop resistance to prescribed anticancer agents over time, leading to treatment failures and tumor relapses.[1,2] Altogether, there remains an unfulfilled need for the development of novel, safe and effective anticancer agents.

Comprising over 2000 species, euphorbia or the spurge family is one of the largest botanical families of flowering plants. Unique characteristics of euphorbia include their milky latex and cyathia, the miniature cuplike inflorescenses, which distinguish them from other plants. Medicinal properties of euphorbia can be traced back to ancient civilizations (reviewed in [3]). For example, the poisonous properties of *E. helioscopia* were known to the Akkadians and Sumerians. Moreover, archives of Greek scholars Hippocrates, Galen and Dioscorides contained the medicinal properties of the latex of *E. resinifera,* referred to as "euphorbium". Traditional medicinal records and modern phytochemical research indicate that euphorbia possess a broad range of attractive pharmacological properties including antimicrobial, anti-inflammatory and anticancer activities.[3-5] The usage of euphorbia in traditional medicine for cancer-related conditions is found in many countries including China[5], Pakistan[6], Brazil[7] and Yemen.[8]

A literature search in the Pubmed database using the search terms "euphorbia" and "cancer" resulted in over 280 hits. Although the majority of these reports relate to *in-vitro* tests such as MTT assay, over 100 publications report the cytotoxic activities of crude extracts from various parts or purified compounds from 60 different species of euphorbia against diverse types of cancers. Over 500 compounds have been isolated from euphorbia, especially terpenoid compounds with diverse core architectures. The chemical structure of these compounds and their biosynthesis are beyond the scope of this review. Readers are referred to an excellent review by Shi et.al. for further information.[4]

Induction of apoptosis of cancer cells by euphorbia-derived substances

The efficacy of anticancer agents is largely attributable to the induction of cell death of cancerous cells, mostly through apoptosis (reviewed in [9-11]). Cells undergoing apoptosis manifest characteristic morphological changes including cell shrinkage, membrane blebbing and chromatin condensation.[12] The induction of apoptosis is mediated by two distinct but related pathways: the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway (reviewed in [10, 12, 13]). The extrinsic pathway is engaged in the activation of death receptors (such as Fas receptor) by its cognate ligands (such as Fas ligand), resulting in the recruitment and formation of death-inducing signaling complex (DISC) and an activation of Caspase-8. On the other hand, the intrinsic pathway is triggered by non-receptor stimuli e.g. growth factor withdrawal, loss of apoptotic suppression by hormone or cytokine signaling, endoplasmic reticulum (ER) stress, free radicals, radiation, and

hypoxia, which lead to the permeabilization of the outer membrane of mitochondria and the release of cytochrome *c*, normally sequestered within the mitochondria, into the cytoplasm. Mitochondrial membrane integrity and the release of cytochrome *c* are regulated by the balance between the pro-apoptotic and anti-apoptotic B-cell lymphoma 2 (Bcl-2) proteins. Pro-apoptotic Bcl-2 proteins include Bax, Bak and Bad, while anti-apoptotic Bcl-2 proteins are Bcl-2, Bcl-xL, and Mcl1. In the cytoplasm, the released cytochrome *c* then triggers the formation of apoptosome and facilitates the activation of Caspase-9. Eventually, the extrinsic and intrinsic pathways converge on the execution pathway - the activation of other caspases, particularly Caspase-3, and the subsequent degradation of cellular components through their protease activities, DNA fragmentation and elimination of apoptotic cells through phagocytosis.

The majority of euphorbia-derived substances induce cancer cell death through the intrinsic pathway of apoptosis.

The apoptosis-inducing activities of crude extracts and purified compounds from euphorbia have been observed in diverse types of cancer cell lines. For example, 7,13-diacetyl-5-angeloyl-20-nicotinyl-3-propionyl-1,2,6,7-tetrahydroingenol (DANPT) (Figure 1A), a tetrahydroingenol diterpene from *E. erythradenia*, induces a cell cycle arrest at G2/M phase in human melanoma cell lines A375 and HMCB, and initiates apoptosis through the intrinsic pathway as supported by an up-regulation of Bax, a down-regulation of Bcl-2 and an increased Caspase-3 activity.[14] Additionally, treatment of non-small cell lung cancer cell line A549 with deoxy Euphorbia factor L1 (Lathyrol-3-phenylacetate-5,15-diacetate) (Figure 1B) or Euphorbia factor L2 (Figure 1C), both of which are lathyrane diterpenoid isolated from the seeds of *E. lathyris* L., is accompanied by an increase in intracellular reactive oxygen species (ROS), a release of cytochrome *c*, activation of Caspase-3 and Caspase-9 as well as PARP cleavage.[15,16] Further, 13-Oxyingenol dodecanoate (13OD) (Figure 1E), a derivative of ingenol isolated from Chinese traditional medicine *E. kansui*, inhibits proliferation, stalls the cell cycle at G2/M phase and induces apoptosis of leukemic cell line K562 through the intrinsic pathway.[17] 13OD treatment results in a decrease of total AKT and phosphorylated mechanistic target of rapamycin (mTOR), indicative of the inactivation of the pro-survival Akt/mTOR pathway and an increase in phosphorylated form of extracellular signal–regulated kinases (ERK) and c-Jun N-terminal kinase (JNK), indicative of an activated MAPK pathway.[17] Other compounds and crude extracts which induce apoptosis of cancer cells through the mitochondrial/intrinsic pathway include jatropha-6(17),11E-diene compounds (Figure 1F) from *E. osyridea*[18], a 6(17)-epoxylathyrane diterpene aellinane (Figure 1G) from *E. aellenii*[19], the diterpenoid Jolkinolide A (Figure 1H) and Jolkinolide B (Figure 1I) from the roots of *E. fischeriana* Steud[20, 21], Euphorbia factor L3 (Figure 1D) from *E. lathyris*[22], 12-deoxyphorbol 13-palmitate (Figure 1F) from the root of *E. fischeriana*[23], a casbene diterpenoid Pekinenin E (Figure 1M) from *E. pekinensis*[24], crude extract of *E. formosana*[25] and crude extract of *E. helioscopia* L.[26]

Some euphorbia-derived substances act through the extrinsic pathway to induce apoptosis of cancer cells.

Although the majority of euphorbia-derived substances induce apoptosis through the intrinsic/mitochondrial pathway, several reports have suggested the involvement of the extrinsic apoptotic pathway in euphorbia-mediated cell death. For example, polyphenols from *E. supina* (PES), also known as Korean prostrate spurge, induce apoptosis of leukemic cell line as evidenced by an increased PARP cleavage; an activation of Caspase-3 and -9; an up-regulation of pro-apoptotic proteins Bax and Bad; a down-regulation of anti-apoptotic proteins Bcl-2, Bcl-xL, and inhibitors of apoptosis (IAP) proteins; and

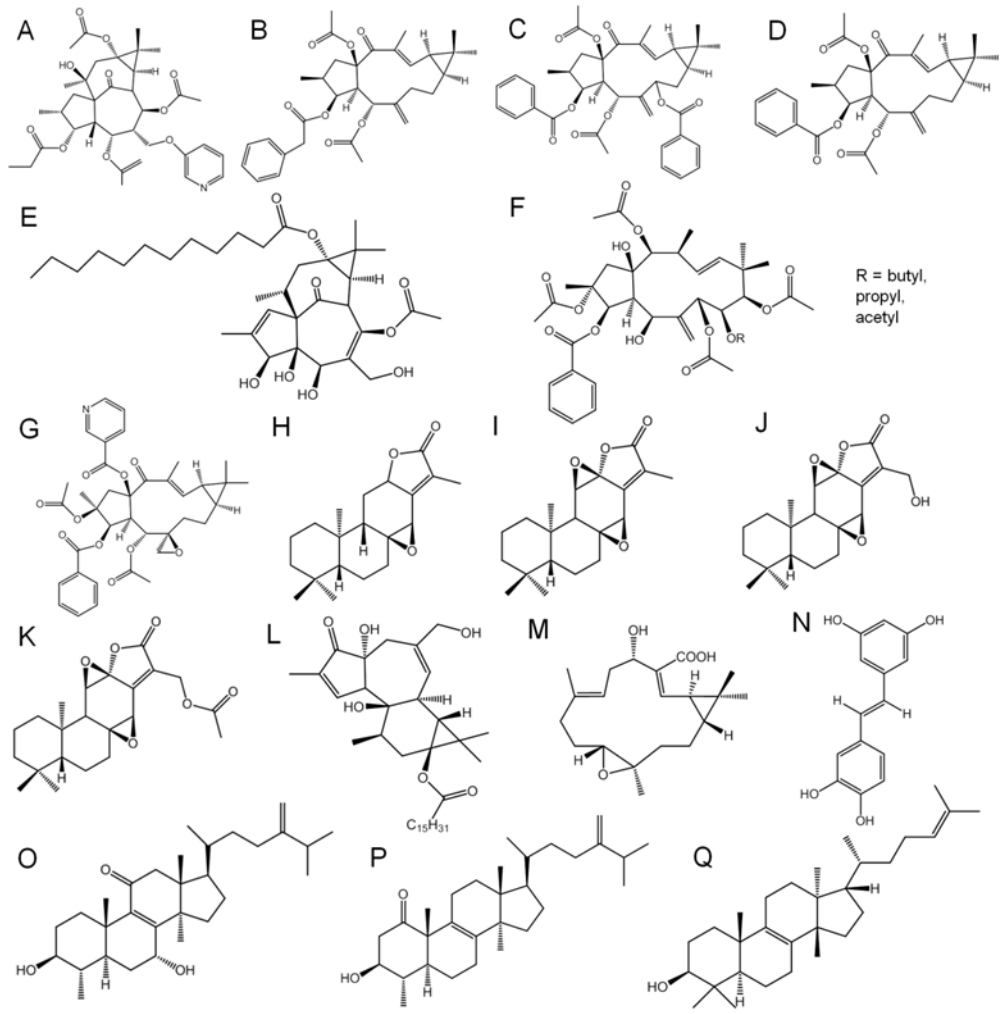

Figure 1 Chemical structures of euphorbia-derived compounds exhibiting pro-apoptotic activities. (A) DANPT (B) Deoxy Euphorbia factor L1 (C) Euphorbia factor L2 (D) Euphorbia factor L3 (E) 13OD (F) Jatropha-6(17),11E-dienes (G) Aellinane (H) Jolkinolide A (I) Jolkinolide B (J) 17-hydroxy-jolkinolide B (K) 17-acetoxy-jolkinolide B (L) 12-deoxyphorbol 13-palmitate (M) Pekinenin E (N) Piceatannol (O) 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-11-one (P) 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-1-one (Q) Euphol

cytochrome *c* release, consistent with the activation of the intrinsic pathway.[27] Additionally, PES-mediated apoptosis is associated with a down regulation of c-$FLIP_L$ and an up-regulation of Fas receptor, indicative of an activation of the extrinsic pathway. The author proposes that PES-mediated apoptosis results from an inhibition of PI3K/Akt signaling pathway and an activation of ERK/MAPK pathway.[27] In support of this notion, PES-mediated apoptosis is potentiated by a concomitant treatment with PI3K/Akt inhibitor, and suppressed by ERK inhibitor.[27] Interestingly, JNK inhibitor and p38 inhibitor elicit no effect on PES-mediated apoptosis.[27] Given that ERK, JNK and p38 are all MAPK proteins, PES appears to exhibit selectivity toward ERK, highlighting PES as attractive candidates for selective ERK activator development. Another euphorbia-derived substance which potentially induces apoptosis through the extrinsic pathway includes a crude extract of *E. esula* as supported by an activation of Caspase-8 in human gastric carcinoma cell line SGC-7901.[28]

Intriguingly, the latex of *E. antiquorum* (EAL) induces apoptosis of cervical cancer cell line HeLa through both intrinsic and extrinsic pathways.[29] Molecular analysis reveals that phosphorylated form of JNK increases upon EAL treatment. Moreover, the activation of pro-apoptotic proteins including Caspase-8 and -9 mediated by EAL treatment is abrogated by a co-treatment with a small molecule inhibitor of JNK, suggesting that apoptotic activity of EAL is mediated, at least in part, through the phosphorylation of JNK.[29] Additionally, EAL treatment is accompanied by an upregulation of ataxia-telangiectasia mutated (ATM) kinase, a master regulator of DNA damage response and a negative regulator of cell cycle progression[10], and its target protein checkpoint kinase 2 (CHK2), suggesting that EAL-mediated cell cycle inhibition likely involves DNA damage.[30] Other euphorbia-derived substances with reported apoptosis-inducing activity through both intrinsic and extrinsic pathways include piceatannol (trans-3,4,30,50-tetrahydroxystilbene) (Figure 1N), a polyphenol found in the seeds of *E. lagascae*[31] and crude extract of *E. formosana* root[32], which induce apoptosis of prostate cancer cell lines DU145 and leukemic cell line THP-1, respectively, and n-hexane extract of *E. lunulata* Bunge which induce apoptosis of gastric cancer cell line SGC7901/ADR[33], and hexane extract of *E. microciadia*, *E. heteradenia*, and *E. osyridea* which induce apoptosis of cervical cancer cell line HeLa, myelogenous leukemia cell line K562 and bladder cancer cell line Fen, respectively.[34]

Other euphorbia-derived substances which induce apoptosis of cancer cells and susceptible cancer cell lines include 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-11-one (Figure 1O) and 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-1-one (Figure 1P) isolated from *E. sogdiana* Popov[35] and crude extract of *E. hirta*[36] against breast cancer cell line MCF7; crude extract of *E. umbellata* (Pax) Bruyns against leukemic cell line Jurkat[37]; crude extract of *E. cheiradenia* against leukemic cell line K562 and Jurkat[38]; macrocyclic diterpenes isolated from *E. erythradenia* Bioss. against ovarian cancer cell lines CAOV-4 and OVCAR-3 and bladder cancer cell line EJ-138[39]; terpenoid compounds from *E. lagascae* against mouse lymphoma cell line L5178 overexpressing human *MDR1* gene [40]; crude extract of *E. mauritanica* against lung cancer cell lines A549 and Lsqr-1[41]; and ethyl acetate extract of *E. helioscopia* against hepatocellular carcinoma cell line SMMC-7721.[42] However, it remains to be determined whether these compounds trigger apoptosis of cancer cells through the intrinsic or the extrinsic pathway. The structurally elucidated compounds from euphorbia with pro-apoptotic activities against cancer cells and their mechanism of action are summarized in Table 1.

Molecular targets of euphorbia-derived substances

Although ample evidence supports the antiproliferative activity and induction of cancer cell death of euphorbia-related substances, the information pertaining to direct molecular targets and underlying mechanisms is in great scarcity. However, a few reports, particularly those related to the characterization of anticancer effect of Jolkinolide B (JB), a diterpenoid compound isolated from *E. fischeriana*, and its derivatives, have begun to shed light on the protein targets and associated mechanisms governing the antiproliferative and pro-apoptotic activities of euphorbia-derived compounds. JB induces apoptosis of leukemic cell line U937[43] and breast cancer cell lines MCF7[44] and MDA-MB-231.[45] JB treatment is associated with a decreased in phosphorylated form of PI3K[44], Akt[43-45] and mTOR[44] as well as an upregulation of phosphatase and tensin homolog (PTEN)[44], consistent with an inhibition of the PI3K/Akt/mTOR signaling pathway. Additionally, JB treatment also down-regulates IAP proteins which are negative regulators of apoptosis.[43] Although the direct molecular target of JB remains unknown, these data indicate that the mechanism of JB-mediated apoptotic induction may be at least partly

Table 1 A list of structurally elucidated compounds with pro-apoptotic activities from euphorbias.

Plant of origin	Compound name	Pro-apoptotic mechanism	Ref.
E. erythradenia	DANPT	- Up-regulate Bax - Down-regulate Bcl-2 - Increase Caspase-3 activity	14
E. lathyris	Deoxy Euphorbia factor L1	- Cytochrome *c* release - Increase Caspase-3 and -9 activity	15
	Euphorbia factor L2	- Cytochrome *c* release - Increase Caspase-3 and -9 activity - Increase cleaved PARP	16
	Euphorbia factor L3	- Loss of mitochondrial membrane potential - Cytochrome *c* release	22
E. kansui	13OD	- Inactivate Akt/mTOR pathway - Activate ERK/JNK/MAPK pathway	17
E. osyridea	Jatropha-6(17),11E-dienes	- Increase Caspase-3 and -9 activity	18
E. aellenii	Aellinane	- Up-regulate Bax - Down-regulate Bcl-2 - loss of mitochondrial membrane potential - Activate caspase 6	19
E. fischeriana	Jolkinolide A	- Up-regulate Caspase-9 - Inhibit PI3K/Akt/mTOR pathway	20
	Jolkinolide B	- Inhibit PI3K/Akt/mTOR pathway - Down-regulate IAPs	43-45
	17-hydroxy-jolkinolide B	- Inhibit JAK-STAT signaling	46
	17-acetoxyjolkinolide B	- Inhibit NF-κB signaling	47
	12-deoxyphorbol 13-palmitate	- Increase Caspase-3 and -9 activitty - Up-regulate Bax, p53, p21 and IκB - Down-regulate Bcl-2 and NF-κB	23
E. pekinensis	Pekinenin E	- Up-regulate C/EBP homologous protein (CHOP) - Increase cleaved Caspase-9 and cleaved PARP	24
E. lagascae	Piceatannol	- Increase cleaved caspase-8, -9, -7, and -3 and cleaved PARP	31
E. sogdiana	3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-11-one	- Increase Caspase-6 activity	35
	3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-1-one	- Increase Caspase-6 activity	35
E. tirucalli	Euphol	- Activate ERK/MAPK pathway	48

explained by the down-regulation of the anti-apoptotic IAPs and the inhibition of pro-survival PI3K/Akt signaling pathway. Additionally, 17-hydroxy-jolkinolide B (Figure 1J), a derivative of JB, inhibits the Janus kinase (JAK) by covalently crosslinking members of the JAK family including JAK1, JAK2 and TYK2 in dimer form through the thiol groups of cysteine residues, interferes with the JAK-STAT signaling, elicits growth-inhibitory effects and induces apoptosis of cancer cell lines.[46] Another related compound, 17-acetoxyjolkinolide B (17-AJB) (Figure 1K), inhibits NF-κB signaling and induces apoptosis of cancer cells.[47] 17-AJB treatment results in an increase in phosphorylated form of IKK-β, which resembles the molecular changes associated with an activation of NF-κB signaling pathway[49], however the kinase activity of IKK-β is suppressed rather than activated in the presence of 17-AJB. The inhibitory effect of 17-AJB is observed for at least 4 hours after wash-out and reversible by a reducing agent DTT. In line with the suppressed activity of IKK, 17-AJB treatment prevents NF-κB nuclear translocation and transcription of NF-κB target genes. Although the mechanism of 17-AJB and IKK-β interaction remains inconclusive, these data indicate that (1) 17-AJB does not interfere with upstream kinases of IKK-β, and (2) the inhibitory effect of 17-AJB likely involves a direct interaction of 17-AJB with IKK which results in the formation of covalent bonds susceptible to reduction.[47] Furthermore, euphol (Figure 1Q), a euphane-type triterpene alcohol isolated from *E. tirucalli*, induces apoptosis of gastric cancer cell lines. An increase in ERK1/2 phosphorylation is detected in cells treated with euphol, suggesting that euphol treatment leads to an activation of ERK/MAPK pathway.[48] Moreover, a small molecule inhibitor of ERK partially decreases euphol-mediated apoptosis induction, indicating that euphol acts through an activation of ERK/MAPK pathway to induce apoptosis in gastric cancer cells.[48] Nevertheless, direct targets of euphol remain elusive. Together, these reports have independently identified kinase enzymes of prominent signaling pathways commonly deregulated in cancer as direct targets or at least as key mediators of growth-inhibitory effects conferred by euphorbia-derived compounds.

Emerging growth-inhibitory mechanisms of euphorbia: interference with metabolic homeostasis of cancer cells

Jolkinolide B (JB) induces apoptosis of mouse melanoma cell line B16F10, as supported by a compromised mitochondrial membrane potential, an increase in ROS level and a change in the apoptosis-related genes Bax, Bcl-2, and Caspases.[21] Interestingly, JB treatment is associated with a decrease in intracellular ATP level and lactic acid production, indicative of a change in energy homeostasis. This may be explained by a decrease in mRNA level of glucose transporters Glut1, Glut3 and Glut4, and glycolysis-related kinases LDHA and Hk2 associated with JB treatment.[21] These findings suggest that, aside from the mechanisms previously discussed, JB may also induce apoptosis of cancer cells through a modulation of cellular metabolism and energy status. Given that cancer cells are known to have aberrant metabolic pathways, also known as the Warburg effect, with a key characteristic of increased in glycolysis which confers energetic surplus and increased supplies of macromolecules as building blocks sustaining the heightened proliferation of cancer cells[50], compounds targeting this pathways hold great promises for the management of a broad range of cancer types. It would be interesting to analyze the relationship between JB-mediated apoptosis and an alteration of genes related to metabolic status of cells in a time-course experiment. Moreover, the effect of JB on transcription factors regulating these altered genes should be explored.

Future perspective

Over the years, research into the bioactivity of euphorbia has revealed the cytotoxic activities of

this unique group of plants against many types of cancer. Given that this activity has been detected in many species from different geographical locations, it is tempting to speculate that the cytotoxic/ anticancer activity may be shared among euphorbia. Crude extracts and a few isolated compounds from certain euphorbia exhibit pro-apoptotic activity against cancer cell lines, as discussed in this manuscript, as well as other attractive anticancer properties such as antimetastatic, antiangiogenic and immunomodulatory effects, which are beyond the scope of this review. Altogether, these reports highlight euphorbias as attractive candidates for the screening and development of novel anticancer leads. Further research should aim to expand on the established cytotoxic effects in terms of (1) their underlying mechanisms, (2) verifying if the effect can be extended toward other cancer types and (3) isolation and characterization of the active compounds or standardization of extracts, particularly for those euphorbia-derived substances with strong effects. Additionally, data mining looking into the correlation between responses and underlying genetic events and/or altered signaling pathways between susceptible and resistant cancer cell lines can be performed, which may suggest potential pathways or molecular targets acted upon by euphorbia-derived substances, as is the case for the identification of PKCδ as a target for ingenol angelate.[51] Notably, euphorbias are generally regarded as toxic, but so are other routinely prescribed chemotherapeutic agents. The true challenges in the clinical usage of euphorbia-derived substances therefore lie in the identifying the therapeutic window in which the substances may be used effectively, with acceptable levels of toxicity. Additionally, the toxicity and efficacy profile of euphorbia-derived substances may be further adjusted through the chemical derivatization of the compounds and/or formulation of these substances into various forms of targeted delivery systems.

Summary

Led by their traditional medicinal uses in diverse cultures, modern phytochemical research has revealed that euphorbia possess many attractive anticancer activities including pro-apoptotic, antimetastatic, antiangiogenic and immune-modulatory effects. Among these activities, the pro-apoptotic effects of euphorbia are most extensively characterized. Given that pro-apoptotic effect is often used to assess the efficacy of anticancer agents, we aimed to summarize the current information regarding the apoptosis-inducing activities of euphorbia. Furthermore, the emerging pro-apoptotic mechanisms of certain compounds, as well as unanswered questions and future prospects of research and development of potential novel anticancer agents have been outlined. All in all, through this review, we hope to facilitate future research into the anticancer activities of this evolutionarily successful group of flora.

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