

Genus *Vernonia*(Asteraceae): a promising source of antitumor agents with pharmacological potentials

ABSTRACT

Antitumor research leads to the development of new molecules that act specifically in tumor cells by blocking or inhibiting their molecular targets. New therapeutic approaches for the screening of bioactive compounds present in medicinal plants have received increasing attention due to their chemopreventive and chemotherapeutic properties. In ethnomedicine, plants of the genus *Vernonia*(Asteraceae) are widely used and some have shown several and interesting biological activities, including anticancer. This present study aimed to document experimental evidence supporting the claimed ethnomedical uses of *Vernonia* species for the treatment of various types of cancer and also to confirm the anticancer potential of these plants. The compounds isolated from aqueous and alcoholic extracts, as well as fractions from different parts of *Vernonia* plants have acted as potential anticancer agents that inhibited the proliferation of various types of human cancer cell lines, including cervical cancer cells, melanoma cells, promyelocytic leukemia cells, breast adenocarcinoma cells, ovarian cancer, liver cancer cell, and human lung cancer cells. Studies have correlated the antitumor activity of *Vernonia* plants by inducing apoptosis and modulating mitochondrial signaling pathways controlled by NF- κ B, Bcl-2 and p53, as well as inducing DNA damage and arresting the cell cycle at the S-phase checkpoint by oxidative stress. In conclusion, *Vernonia* species act as a promising source for drug development. However, further studies are needed to explore the exact mechanism of action, pharmacokinetics, chronic toxicological studies, safe dose consumption, and possible interactions with other herbs.

Keywords: *Vernonia* genus, anticancer, chemotherapeutic, medicinal plant.

1. INTRODUCTION

Cancer is the foremost cause of deaths around the world, and the expectation from 2040 is that there will be a 47% increase cancer cases, due to risk factor associated with globalization and a growing economy [1]. "Usually, the cancer therapy is based in radiotherapy and chemotherapy, and are accompanied from side effects such as nauseous, vomits, loss of appetite, infertility, acute and chronic renal insufficiency and electrolytes disturbances"[2,3]. "The multiple drug resistance (MDR) in chemotherapy is other big problem in cancer treatment, a phenomenon where by tumors that acquire resistance to one

type of therapy are resistant to several other drugs, that are often quite different in both structure and mode of action" [4-5]. "The mechanism of MDR can involve the inhibition of drug transportation through the P-glycoprotein and other transporters, inhibition of drug-induced apoptosis and proliferation of tumor stem cells with a MDR phenotype. These factors can be explaining the high mortality and morbidity in patients with advanced cancer, when the chemotherapy shows low efficacy in eradicate cancer cells without damaging the normal cells"[4,5,6].

The research of new approaches for treatment of cancer is essential for health of patients, and the natural products may offer a range of new chemical compounds with anticancer properties [6]. Medicinal plants have been used for millennia by humanity to treatment for diseases, and are the main source of chemical compounds for many types of therapy. These plants were passed down through generations, of selected healers in community, and the knowledge obtained is the result of the experience accumulated [7].

Several plants can have metabolites with potent biological activities, such as anti-inflammatory, antioxidant, immunomodulatory, and antitumor. These natural compounds may create an unfavorable environment to cancer cells growth, assisting on the regular treatment efficacy and reducing the side effects [8,9]. The role of anticancer properties of medicinal plants can be included inhibition cancer cell-related activating proteins, signaling apoptotic pathways, and enzymes such as topoisomerase, and cyclooxygenase, activating DNA repair mechanisms, and antimetastatic effects [6,10,11].

"*Vernonia* (Asteraceae) is the largest genus in the Vernoniae tribe with close to 2000 species. *Vernonia* species grow in a wide range of habitats of broad ecological diversity and climatic conditions including tropical forest, marshes and wet areas, dry plains, tropical savannahs, desert xeric or dry sites and frosty regions"[12]. "*Vernonia* species are frequently used in ethnomedicine for treatment of diabetes, malaria, tuberculosis, hyperlipidemia, hypertension, chronic coughs, and fever"[13,14,15]. They are also used to strength immune system, preventing to cancer and other diseases, due to potent antioxidant agents [9].

"Regarding the phytochemical composition, the most abundant compounds in crude extracts and fractions isolated of the genus *Vernonia* are sesquiterpene lactones and flavonoids, which are the compounds with the greatest interest antitumor potential within the *Vernonia* species"[16,17,18]. The antineoplastic potential of this compounds have been showed on a range of studies *in vitro* on diverse tumor cells lines, inducing antiproliferative effects by modulation of apoptosis pathways[5,11]. In this work, we explored the claimed ethnomedical effect of *Vernonia* genus by anticancer therapy, through the documentation of experimental evidences that supporting this antitumor potential.

2. ETHNOBOTANY AND ETHNOMEDICINE OBSERVATIONS

"Ethnobotany has proven to be a powerful tool to document, value, and understand how different traditional people relate to natural resources, especially plants of medical and pharmaceutical interest"[19]. "A plant is considered medicinal when it is used in the reversal of different health conditions, and when this effect is attributed to the action of their constituents whose activity can be scientifically proven" [20].

"The diverse medicinal plant sources, commonly used in infusions, ointments, patches, compresses and lotions, have demonstrated their curative potential, giving rise to information that was transmitted orally between the generations"[21]. "In fact, local and traditional knowledge has been compiled into potentially interesting historical texts for pharmaceutical research, especially with regard to cancer therapies"[22,23].

“Asteraceae is the largest family of angiosperms, with 1700 genera and 24,000 species widely distributed around the world. A vast majority of plants in this Family are herbaceous, while trees and shrubs are comparatively rare”[24]. “Asteraceae species have been widely used in folk medicine for several therapeutic purposes”[25], and “are very common in different regions of Brazil, mainly in the Cerrado (savanna) biome, where this family is well represented by the approximately 250 genera and more than 2000 species”[26].

“Plants of the *Vernonia* genus (Asteraceae) occur in different habitats ranging from tropical forests, marshes, savannahs, dry plains, desert areas to frosty areas”[27]. “The compounds found in species of this genus have been usually used in popular medicine to cure a wide spectrum of disorders including asthma, sores, inflammatory swellings, skin ailments, and kidney troubles”[28]. “The bioactive phytochemicals present in *Vernonia* plants include tannins, lignans, coumarins, quinones, stilbenes, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins, and proanthocyanins. However, it is important to say that those same compounds also can cause adverse issues for human health, if bad administered. These plants are widely used in ethnomedicine and some have demonstrated bioactivity in antimicrobial, anti-inflammatory, analgesic, antimalarial, and anticancer assays”[29,30,31,32].

Species of *Vernonia* are gaining global attention as a possible anticancer agent. For example, the study by Toyang et al. [33] revealed that “the crude extract of the root tubers of *Vernoniaguineensis* and pentaisovaleryl sucrose isolated from this plant exhibited *in vitro* cytotoxicity and clonogenic activity against the PC-3 and DU145 prostate cancer cell lines as well as *ex-vivo* antiangiogenic activity. Previous studies showed that *Vernoniaamygdalina* aqueous extract (VA) retards the proliferation of ER+ and ER- breast cancerous cells”[34], and Opata and Izevbigie [35] showed that “aqueous extract of VA alters cell membrane permeability and efflux in breast cancer cells”. Furthermore, Turak and Aisa[36] reported that “elemanolides and sesquiterpenoids isolated from *Vernoniaantheilmintica* seeds extract showed a strongly inhibitory effect against human colon cancer (HCT-15) and PC-3 cell lines”.

“The utility of natural products is to provide an original source of novel structures aiming at medicine discovery, not necessarily as the final drug entity, but as sources of novel and peculiar molecules to be used for semi-synthetic drugs or templates for totally synthetic one”[37]. “In this context, plants with antiproliferative activity are of great interest because cancer is one of the leading causes of death and globally, the numbers of cases of cancer are increasing gradually. Currently, many difficulties and challenges persist in cancer therapy such as drug resistance, toxicity and low specificity of drugs”[38]. “Extensive research with natural products has been carried out to characterize the molecular mechanisms of anticancer activities and day-by-day the anticancer property of various plants is being identified”[39].

3. VERNONIA GENUS ANTITUMOR ACTIVITY

“Antitumor research is a very active field that has generated a large amount of information on clinical aspects of chemotherapy”[40]. “New therapeutic approaches for the screening of bioactive compounds present in medicinal plants have received increasing attention due to their chemopreventive properties activities”[41].

Sesquiterpene lactones [42], flavonoids [3], steroids [43], and polysaccharides [33] have been detected in preparations from different species of *Vernonia*, and several biological activities were reported, including antitumor activity. “The anticancer property of these

compounds has attracted a great deal of interest and extensive research studies have been carried out to characterize the anticancer activity, the molecular mechanisms, the chemopreventive potential and the chemotherapeutic application of these compounds” [3,44,45]. Studies focusing on anticancer activity of *Vernonia* species are summarized in Table 1.

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Table 1. Vernonia plant parts tested experimentally *in vitro* or *in vivo* for cancer treatment.

	Source tissue	Preparation	Phytochemical	Type of cancer cell line/tumor treated	Reference
<i>Vernonia amygdalina</i>	leaves	methanolic extract	vernoniamyosides A–D, vernoamyoside D, and vernonioside B2	prostate cancer (PC-3)	[46]
			apigenin, and luteolin(3',4', 5,7tetrahydroxyflavone	breast cancer (4T1)	[11]
<i>Vernonia anthelmintica</i>	seeds	ethanol extract	vernodalidimers C, -D, and -E	breast cancer (T47D)	[73]
			cynaropicrin	colon cancer (HCT-15)	
			vernonilide A	prostate cancer (PC-3)	[36]
	dried fruits	ethyl acetate fraction of ethanol extract	isorhamnetin	pancreatic cancer (PANC-1)	[75]
<i>Vernonia calvoana</i>	leaves	VC fraction 7	flavonoids	ovarian cancer (OVAR-3) cells	[3]
<i>Vernonia cinerea</i>	dried aerial part	crude ethanol extract	8 α -tigloyloxyhirsutinolide-13-O-acetate	oral squamous cell carcinoma (HSC4) and lung carcinoma (A549)	[51]

<i>Vernonia condensata</i>	leaves	aqueous extract	No study	leukemic cells (Reh, Nalm6, K562 Molt4), breast cancer cell (MCF7) and human embryonic kidney (HEK293T)	[54]
<i>Vernonia divaricata</i>	leaves	crude methanol extract	steroids, esters, triterpenoids and glycosides	MCF-7 (breast), HL-60 (leukaemia) and the PC-3 (prostate) cancer	[43]
<i>Vernonia extensa</i>	dried aerial part	dichloromethane fraction of extract	vernodalidimer L	liver cancer (HepG2)	[44]
<i>Vernonia guineensis</i>	Root	root extract (VGDE)	pentaisovaleryl sucrose	prostate cancer (PC-3)	[33]
<i>Vernonia scorpioides</i>	leaves	dichloromethane fraction of leaf extract	hirsutinolides and glaucolides	Ehrlich's tumor, sarcoma 180 ascitic tumor, melanoma (B16-F10)	[65,69,72]
		ethyl acetate extract	hirsutinolides and glaucolides	color cancer (HCT-116) and ovarian carcinoma (OVCAR 8)	[71]
<i>Vernonia zeylanica</i>	leaves	ethyl acetate extract	vernolactone	human embryonal carcinoma (NTERA-2)	[42]

Among species of *Vernonia*, antitumor properties have been described *Vernonia amygdalina* [5,11,47,48,49], *Vernonia bockiana* [50], *Vernonia cinerea* [51,52,53], *Vernonia condensata* [54], *Vernonia divaricata* [43], *Vernonia extensa* [44], *Vernonia fimbriifera* [55], *Vernonia glaberrima* [56,57], *Vernonia kotschyana* [58], *Vernonia leopoldii* [59], *Vernonia mespilifolia* [60], *Vernonia pachyclada* [61], *Vernonia paniculata* [62], *Vernonia patula* [63], *Vernonia polyanthes* [64], and *Vernonia scorpioides* [65,66,67], *Vernonia zeylanica* [42,68].

“Previous studies were carried out with the fractions and sub fractions of the *V. scorpioides* leaf until the isolation of its sesquiterpene lactone. The antitumor activity study was carried out in mice with Ehrlich ascitic and solid tumors where the dichloromethane sub fraction totally reduced the ascites volume and the number of tumor cells in the peritoneum of the mice at 5 mg/kg body weight” [46]. “The treatment with ethyl acetate sub-fraction extracted from the dichloromethane sub-fraction eliminated sarcoma 180 ascitic tumor in mice” [69].

The results obtained in the studies by Mbemi et al. [3] demonstrated that “*Vernonia calvoana* (VC) fraction 7 significantly reduced the percentage of live cells in a dose-dependent manner, suggesting its antiproliferative effect against ovarian cancer (OVCAR-3) cells. Other *Vernonia* species including *V. divaricata* and *V. amygdalina* act as potential anticancer agents that inhibit the proliferation of breast cancer cells, human leukemia (HL-60) cells, breast (MCF-7) cells and prostate (PC-3) cells” [11,43].

Toyang et al. [14] isolated “vernopicrin and vernomelitensin for the first time from *Vernonia guineensis* leaf acetone extract. These compounds exhibited activity against all the 10 cancer cell lines tested with IC₅₀ values ranging from 0.35 to 2.04 μM and 0.13–1.56 μM respectively. The A375 cell line was the most sensitive cell to both compounds, while the A549 and MCF-7 cell lines were least sensitive to vernopicrin and vernomelitensin, respectively. In addition, the authors demonstrated the *in vivo* antitumor activity of the root dichloromethane extract of *V. guineensis* (VGDE) against the PC-3 prostate tumor. The absence of any noticeable signs of toxicity in the treated mice is also an indication that the VGDE extract can be considered to be reasonably safe. The authors suggested that the pentaisovaleryl sucrose obtained from the dichloromethane extract may be responsible for the *in vivo* activity observed. This study provides preliminary *in vivo* validation of the use of preparation soft his plant in folk medicine to manage prostate related problems” [70].

“Vernodalin and vernolepin isolated of the CH₂Cl₂ extract from *V. extensa* aerial parts were very attractive compounds since they exhibited strong cytotoxicity and high cancer-selectivity towards the panel of ten cancer cell lines. They showed selective toxicity to leukemia cancer cells, HL-60 and acute T lymphoblastic leukaemia (MOLT-3) with the IC₅₀ values of 2 and 5 being 2.28 and 3.83 μM in HL-60 cells and 3.92 and 5.29 μM in MOLT-3 cells, respectively” [44].

“The extract from *V. scorpioides* in ethyl acetate showed a high antiproliferative activity for two of the three human tumor cell lines used (HCT-116 and OVCAR-8)” [71]. “Previous *in vivo* studies have demonstrated its high antitumor potential” [46]. “Literature data indicated that sesquiterpene lactones (hirsutinolides and glaucolides) isolated from leaves and flowers of *V. scorpioides* have a cytotoxic effect against Hela and cervical cancer cells” [72].

Several reports have been reported on the anticancer potential of *Vernonia anthelmintica* preparations and their isolated compounds. For example, Turaket al. [73] evaluated “isolates of ethanol extract for their cytotoxic abilities against colon cancer (HCT-15), lung carcinoma (A-549), PC-3, and human breast cancer (T47D) cell lines. The authors observed that vernodalidimers -C, -D, and -E showed potent cytotoxicity (IC₅₀: 6, 1, and 13 μM, respectively) against breast cancer (T47D) cells which was more effective than standard,

doxorubicin (IC₅₀: 26 µM). In another study, the cytotoxic effects of vernodalidimers -F, -G, -H, vernonilide -A, -D, -E, -F, cynaropicrin and vernodalol were evaluated against human colon cancer (HCT-15), A-549, PC-3 and cervical cancer cell lines. Vernonilide A strongly inhibited cancer cell lines (IC₅₀: 5, 6, 6 and 8 µM, respectively) while cynaropicrin strongly inhibited HCT-15 (IC₅₀: 1 µM) and PC-3 (IC₅₀: 1 µM) cells"[36,74].

4. MECHANISMS OF ANTITUMOR ACTION

The mechanism underlying the antitumor activity of preparations of *Vernonia* species has been extensively investigated that support their chemopreventive and anticancer properties (Table 2). Wang et al.[75] revealed for "the first time that isorhamnetin, a flavonoid derived from dried fruits of *V. anthelmintica*, has anti-proliferation and migration inhibition activities in PANC-1 pancreatic adenocarcinoma cell line. Isorhamnetin caused cell cycle S-phase arrest through downregulation of cyclin A. In addition, isorhamnetin decreased the phosphorylation levels of MEK and extracellular signal regulated kinases (ERK) in the Ras/MAPK pathway, which is involved in regulating cell proliferation, differentiation and apoptosis. Mitogen activated protein kinases (MAPKs) or ERKs are essential compounds in transduction through their role in modulating gene transcription in the nucleus in response to changes in the cellular environment"[76]. "Activation of ERK is instrumental in normal and aberrant cell growth, including malignant transformation"[77].

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Table 2. Examples of mechanisms involved in the antitumor activity of some *Vernonia* plant compounds.

Plant	Phytochemicals	Type of cancer cell lines/tumor treated	Mechanism of action	Reference
<i>Vernonia amygdalina</i>	vernoniamyosides	prostate adenocarcinoma cells (PC-3 cells)	mitigation of key regulatory patterns of MAPK and pro-tumor transcription factors/proto-oncogenes	[79]
		breast cancer (BT-549)	induce apoptosis by cleavage of a pro-apoptotic molecule BAX, which activates caspase-3 and caspase-9 in breast cancerous cells; decrease mesenchymal stem cells (MCSC) proliferation; and reduce the initiation and progression of MCSC-induced xenografts <i>in vivo</i>	[78]
		4T1 breast cancer cells	induced apoptosis, increased cell accumulation in the G2/M phase of the cell cycle and inhibited intracellular signals such as PI3K and mTOR expression.	[11]
<i>Vernonia anthelmintica</i>	Isorhamnetin	pancreatic cancer (PANC-1)	cell cycle S-phase arrested through downregulation of cyclin A; decreased the	[75]

<i>Vernonia calvoana</i>	flavonoids	ovarian cancer (OVAR-3) cells	phosphorylation levels of MEK and extracellular signal regulated kinases (ERK) in the Ras/MAPK pathway induce DNA damage and cell cycle arrest at the S-phase checkpoint of the cell cycle through oxidative stress	[3]
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“An extensive *in vitro* and *in vivo* data indicated that aqueous extracts from *V. amygdalina* leaves (VA) have therapeutic potential alone or in combination with known anti-breast cancer therapies. VA extracts (1) are inhibitors of the ERK signaling pathway; (2) induce apoptosis by cleavage of a pro-apoptotic molecule BAX, which activates caspase-3 and caspase-9 in triple-negative breast cancerous cell lines of White origins; (3) decrease mesenchymal stem cells (MCSC) proliferation, as well as mammosphere initiating ability *in vitro*; and (4) reduce the initiation and progression of MCSC-induced xenografts *in vivo* (in nude mice)”[78]. These findings, demonstrating VA extracts’ anti-cancer activities and ability to synergize with paclitaxel (Tax), coupled with its chemopreventive actions, provide hope for better cancer treatment, absence of severe side-effects, and improved survival rates for patient with breast cancer.

“Another study also on VA, carried out by Cameron et al.[79], hypothesized that VA would inhibit the proliferative activity of Tax resistant prostate adenocarcinoma cells (PC-3 cells) by mitigation of key regulatory patterns of MAPK and pro-tumor transcription factors/proto-oncogenes. Substantial evidence suggests that MAPK, NF- κ B, c-Myc, and AKT are involved in tumor cell proliferation, survival, and metastasis”[80,81], and “natural compounds that inhibit these factors have shown promise as a mode of chemoprevention”[6,82].

Mbemi et al. [3] demonstrated that “VC fraction 7 (F7) from *V. calvoana* leaves was able to inhibit cell proliferation, induce DNA damage and cell cycle arrest at the S-phase checkpoint of the cell cycle in OVAR-3 cells through oxidative stress, as demonstrated by an increase in malondialdehyde production and a decrease in catalase and glutathione activities in treated cells compared to the control. All these unique properties of VC F7 against OVAR-3 cells strongly suggest that VC F7 may be a potential targeting molecule that may be used as a therapeutic agent for ovarian cancer(Fig. 1)”.

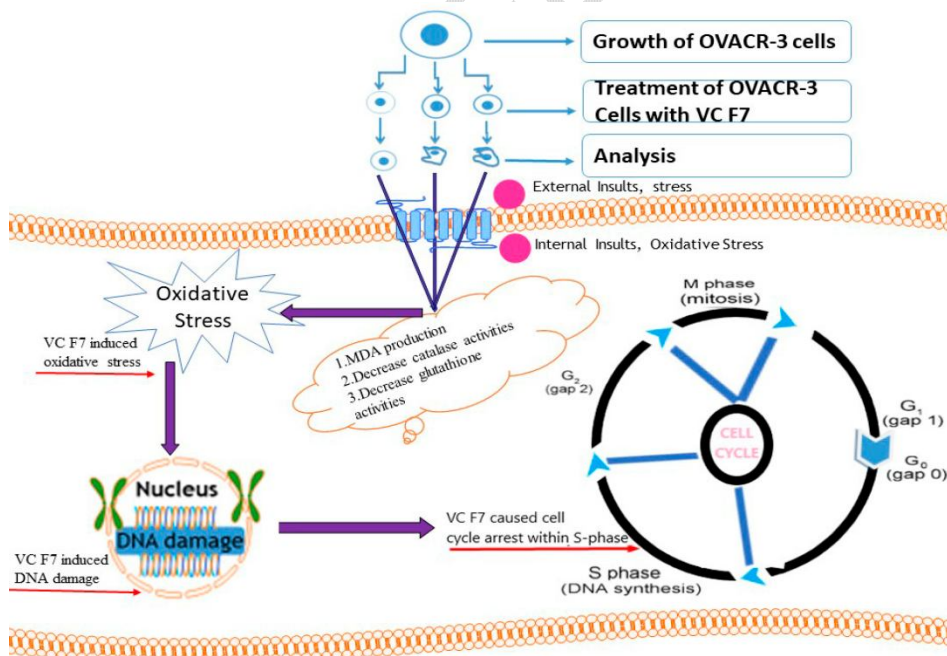


Fig. 1. Mechanism of antitumor action of flavonoids present in *Vernonia calvoana* fraction 7 (VC F7) in human ovarian cancer (OVAR-3) cells. Source: Mbemiet al.[3]

Hasibuan et al. [11] studied “the anticancer effects of *V. amygdalina* leaves extracts on 4T1 breast cancer cells. The preparations induced apoptosis, increased cell accumulation in the G2/M phase of the cell cycle and inhibited intracellular signals such as PI3K and mTOR expression in breast cancer cells. Similarly, vernodalin, vernolepin, and vernolide from the CH₂Cl₂ extract from aerial part of *V. extensa* induced apoptosis on human hepatoma (HepG2) cells in a dose dependent manner and these effects correlated with G2/M phase cell cycle arrest”[44].

Rocha et al. [45] evaluated “the anticytotoxic and antigenotoxic potential of *Vernoniapolyanthes* leaf aqueous extract (VpLAE) and its n-butanol fraction (n-BF) against doxorubicin (DXR), a widely used chemotherapeutic, capable of performing single and double breaks in DNA. The association of DXR with VpLAE or n-BF in the co-, pre-, and post-treatments demonstrated an anticytotoxic potential of *V. polyanthes*. In contrast, the cytotoxicity of DXR was potentiated by VpLAE and its aqueous, n-butanol, and ethyl acetate fractions (0.25–1 mg/mL) on human lymphocytes during co-treatment”[83].

“Doxorubicin is part of one of the most effective groups of antineoplastics used in current clinical practice. However, its use is limited by chronic and acute toxic side effects and susceptibility to numerous drug interactions”[84]. “The antitumor and toxic effects of DXR contribute to the production of free radicals and to the occurrence of oxidative stress”[85]. “In this context, phytochemical compounds have been described as an alternative to mitigate the harmful effects caused by DXR since metabolites from natural products have shown to be promising in overcoming the limitations of DXR in pre-clinical models such as chemosensitizers, chemoresistance inhibitors, and protectors chemotherapeutics in different types of cancer”[86,87].

5. CONCLUSION

In summary, *Vernonia* genus plants could be considered as promising sources for anti-tumor agents with therapeutic potential alone or in combination with known anti-cancer agents. The potential uses of these plants are owing to the presence of phenolic acids, steroids, and terpenes in its composition. Given the knowledge of the possible underlying mechanisms of these bioactive constituents that confirm their potent antiproliferation activity, research should be extended to study the absorption and bioavailability of extracts, fractions, or active moieties responsible for pharmacological actions. In addition, further studies are necessary to conduct acute and chronic studies to understand the potential adverse effects fully.

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