

Phytochemicals, Pathways, Mechanisms and Clinical Applications: A Comprehensive review on *Hedyotis diffusa* (Bai Hua She She Cao) in Cancer Management

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Traditional Chinese medicine has long utilized *Hedyotis diffusa* (Bai Hua She She Cao) for its diverse therapeutic properties, including anticancer potential. The study examines its mechanisms of action, bioactive compounds, and synergistic effects with conventional cancer therapies. A literature review analyzed studies on *Hedyotis diffusa*, focusing on its anticancer effects, including apoptosis induction, metastasis suppression, angiogenesis inhibition, and modulation of molecular pathways like NF- κ B, STAT3, and VEGF. *Hedyotis diffusa* demonstrated significant anticancer activity, targeting key pathways and reducing cancer cell proliferation, metastasis, and angiogenesis. Its bioactive compounds, such as polysaccharides and flavonoids, enhanced chemotherapy and radiation therapy efficacy while mitigating side effects. By integrating traditional and modern medicine, *Hedyotis diffusa* presents a promising complementary cancer therapy. Its clinical applications require more investigation and optimize its role in oncology.

Keywords: Apoptosis induction; Bioactive compounds; Cancer therapy; *Hedyotis diffusa*; Traditional Chinese Medicine.

Cancer, which is defined by the unchecked development and spread of aberrant cells, continues to rank among the world's top causes of mortality. Globally, the incidence and mortality burden of cancer is rising quickly.¹2018 had 18.1 million cancer diagnoses and 9.6 million cancer-related deaths.²Malignancies of the colon, lungs, breast, and prostate are among the most common tumours, whereas malignancies of the liver, stomach, and lungs are among the most lethal. In China, there are 3.6 million new instances of cancer and 2.2 million deaths from the disease each year.³Despite

advances in cancer diagnosis and treatment, managing the disease remains challenging. Radiation, chemotherapy, and surgery are examples of conventional therapies that frequently have serious side effects, such as toxicity, immunological suppression, and a decreased quality of life. Drug resistance and variability in patient responses further limit treatment efficacy, while high costs restrict access, particularly in resource-limited settings. These challenges underscore the need for alternative, cost-effective, and less toxic therapies.⁴Herbal medicine has a long history

of being used to treat cancer because of its anti-inflammatory properties and wealth of anticancer ingredients. It has been demonstrated that these elements affect cancer immunology, the tumour microenvironment, and the course of treatment both directly and indirectly.^{5,6} Natural and herbal remedies have long been used in traditional medicine to treat a range of ailments, including infections and cancer.^{7,8}

In this context, Traditional Chinese Medicine has gained attention for its potential to address these limitations, offering natural, holistic approaches that may enhance therapeutic outcomes and improve patient well-being. Traditional Chinese medicine treats cancer holistically, emphasising the body's natural defences and re-establishing equilibrium (Fig. 1.). Rooted in concepts like Yin-Yang harmony and Qi flow, TCM views cancer as a result of internal imbalances caused by factors such as stagnation, toxins, and deficiencies. Treatment strategies aim to "clear heat," "detoxify," and "invigorate blood circulation," often using a combination of herbal medicines, acupuncture, dietary adjustments, and lifestyle modifications.⁹ Traditional Chinese Medicine herbal formulas target not only the tumor but also the surrounding microenvironment, reducing inflammation,¹⁰ boosting immune function, and alleviating side effects of conventional treatments. This integrative approach has gained attention for its potential to enhance therapeutic outcomes, improve patients' quality of life, and provide personalized care tailored to individual needs.¹¹

Hedyotis diffusa, commonly known as Bai Hua She She Cao, a popular herb in Traditional Chinese Medicine (TCM), *Oldenlandia diffusa* (Willd) Roxb is known for its anti-inflammatory and detoxifying qualities.¹² Traditionally, it has been used to "clear heat" and "remove toxins," aligning with TCM principles for treating conditions like abscesses, infections, and inflammations (Fig. 2).¹³ Over time, its applications have extended to cancer therapy, particularly for digestive system cancers,¹⁴ liver cancer,¹⁵ and lung cancer.¹⁶ The herb is often incorporated into TCM formulations and prescribed alongside other medicinal plants to enhance its efficacy. Packed with beneficial substances including flavonoids, polysaccharides, and terpenoids, *Hedyotis diffusa* exhibits potent

anticancer,¹⁷ anti-inflammatory,¹⁸ and immunomodulating effects. *Hedyotis diffusa* exhibits significant anticancer properties through multiple mechanisms of action. By causing apoptosis and upsetting the cell cycle, its bioactive substances which include flavonoids, polysaccharides, and terpenoids inhibit the growth of cancer cells. The herb also stops metastasis by stopping cancer cells from migrating and invading. *Hedyotis diffusa* also has anti-angiogenic properties, which stop new blood vessels from growing and supplying tumours. By decreasing inflammation and boosting immune responses to cancer cells, it modifies the tumour microenvironment. These mechanisms collectively make it a promising natural agent for combating cancer, with the potential to complement conventional therapies while minimizing side effects. Its holistic approach not only targets cancer cells but also supports overall health, making it a valuable component of integrative cancer care in TCM practices. Investigating medicinal herbs like *Hedyotis diffusa* is vital for overcoming the limitations of conventional cancer treatments. With its proven anticancer properties, such as inducing apoptosis, inhibiting metastasis, and modulating immunity,¹⁹ *Hedyotis diffusa* offers a natural, cost-effective alternative with fewer side effects. Due to its anti-tumor efficacy in animals with tumours and anti-proliferative activity in cancer cells, *H. diffusa* has drawn more and more attention.²⁰⁻²³ It is the most commonly suggested Chinese herb for patients with breast and colon cancer, according to Taiwan's National Health Insurance Research Database.^{24, 25} Research into its mechanisms can enhance current therapies, promote integrative medicine, and improve patient outcomes while advancing sustainable and accessible cancer treatments. This review's goal is to investigate *Hedyotis diffusa*'s potential for therapeutic use (Bai Hua She She Cao) in cancer treatment, focusing on its role within the framework of Traditional Chinese Medicine (TCM).

The scope includes a discussion of preclinical and clinical evidence, its integration into TCM practices, and future directions for research and therapeutic development, emphasizing its promise as a natural, holistic approach to cancer care.

MATERIALS AND METHODS

A comprehensive literature search was conducted using databases such as PubMed, Scopus, Web of Science, and Google Scholar up to 2025. The search utilized the keywords “*Hedyotisdiffusa*,” “cancer,” “anticancer activity,” “mechanism of action,” “toxicity,” “drug interaction,” and “phytochemicals,” with Boolean operators (AND, OR, NOT) to refine the results. Initially, 1,250 articles were retrieved. After removing 200 duplicates and screening titles and abstracts for relevance, 850 articles were excluded due to irrelevance or duplication. Full-text screening was conducted on the remaining 200 articles, resulting in the inclusion of 125 studies based on the inclusion and exclusion criteria. The inclusion criteria encompassed original research articles published in English, focusing on the anticancer effects of *Hedyotisdiffusa* through *in vitro*, *in vivo*, or clinical studies. Exclusion criteria included review articles, editorials, conference abstracts, non-English articles, and studies that did not specifically address cancer or the anticancer mechanisms of *Hedyotisdiffusa*. (Fig.3.), illustrate the research process based on the PRISMA guidelines, which is typically used for systematic reviews.

***Hedyotisdiffusa* (Bai Hua She She Cao) in Traditional Chinese Medicine**

For millennia, Traditional Chinese Medicine (TCM) has made extensive use of *Hedyotisdiffusa* (Bai Hua She She Cao), primarily for its detoxifying and heat-clearing properties. Historically, it has been employed to treat conditions such as infections, abscesses, and inflammations, aligning with TCM theories that associate cancer with heat, toxin accumulation, and blood stagnation. TCM approaches to cancer therapy focus on clearing heat, removing toxins, and promoting blood circulation to restore balance and inhibit tumor growth. Bai Hua She She Cao is commonly prepared as decoctions, powders, or extracts, often combined with other herbs to enhance its efficacy. It is a key component in various TCM prescriptions, such as “Clear Heat and Detoxify Decoction” and “Anti-Toxin Combination,” which are used to manage cancers of the digestive system, liver, and lungs. These formulations are tailored to individual patients,

addressing not only the tumor but also systemic imbalances, making *Hedyotisdiffusa* an integral part of TCM cancer therapy.²⁶

Phytochemical Composition of *Hedyotisdiffusa*

China, Japan, Indonesia, and other parts of the Orient and tropical Asia are home to the annual herb *H. diffusa*.²⁷ Reaching a height of 50 cm, the plant is usually found growing in damp fields and farming ridges. The papilla was seen in the stem’s transverse section, which is glabrescent to glabrous and terete to slightly flattened. The opposite, linear, narrowly elliptic, sessile or subsessile leaves are 1-4 × 0.1-0.4 cm and have a drying membrane. The corolla is white, and the pedicelled blooms are paired in axillary racemes.³⁰ Techniques for distinguishing *H. diffusa* from closely related species, such *Hedyotiscorymbosa*, have been devised, to ensure proper medicinal use. These methods include DNA sequencing,³¹ gas chromatography-mass spectrometer (GC-MS), high performance liquid chromatography (HPLC),²⁹ and thin-layer chromatography (TLC).²⁸ Numerous studies on *H. diffusa* have identified a number of significant phytochemicals, such as iridoids,³² triterpenes,³³ flavonoids,³⁴ anthraquinones,³⁵ phenolic acids,³⁶ and their derivatives, sterols,³⁷ alkaloids,³⁸ volatile oils,³⁹ polysaccharides,⁴⁰ cyclotides,⁴¹ and coumarins,⁴² because of the advancement of analysis technologies like nuclear magnetic resonance–mass spectrometers (NMR-MS), liquid chromatograph–mass spectrometers (LC-MS), and mass spectrometers (MS) (Fig.4).

These compounds work synergistically to target multiple pathways involved in cancer development, including inflammation, angiogenesis, and immune evasion, making *Hedyotisdiffusa* a promising natural therapeutic in cancer treatment. These substances work in a number of ways, such as triggering apoptosis, preventing the growth of cancer cells, and preventing angiogenesis and metastasis. Compared to other cancer-targeting herbs like *Scutellariabaicalensis*, which is abundant in baicalin and wogonin, or *Curcuma longa* (turmeric), known for its curcuminoids, *Hedyotisdiffusa* offers a broader spectrum of bioactive compounds that work synergistically. While *Scutellariabaicalensis* is renowned for its anti-inflammatory and apoptotic effects,⁴³ and *Curcuma longa* excels in inhibiting metastasis and oxidative stress,⁴⁴ *Hedyotisdiffusa* uniquely

integrates immune modulation, detoxification, and angiogenesis inhibition into its mechanisms. This diverse phytochemical composition makes it highly adaptable for use in both standalone and integrative cancer therapies, offering complementary benefits when combined with other natural or conventional treatments. Using a CRC mouse xenograft model, researchers showed the anticancer benefits of *EEHDW* in vivo in 2012. By inhibiting STAT3 phosphorylation, they were able to diminish the tumor's weight and volume. Both in vitro and in vivo, *EEHDW* demonstrated strong anti-colorectal cancer efficacy against HCT-8, HT-29, HCT-116, and SW620 cells. It controlled inflammatory (IL-1 α , IL-6, IL-4, IL-10, TNF- α) and angiogenic (COX-2, iNOS, eNOS, HIF-1 α) factors and downregulated oncogenes (Bcl-2, Bax, Pim-1, p53), affecting tumor angiogenesis, proliferation, and apoptosis (Fig.5 & 6). These effects suggest its role in modulating key signaling pathways, including p38, AKT, STAT3, JNK, and ERK1/2.^{45, 46}

Mechanisms of Action of *Hedyotis Diffusa* in Cancer Therapy

Hedyotis diffusa, a well-known medicinal herb in traditional Chinese medicine, exhibits significant anticancer properties through multiple mechanisms (Fig.5). By controlling important signalling pathways like the PI3K/Akt and MAPK pathways, it induces apoptosis in cancer cells, which results in programmed cell death. This is one of its main mechanisms of action. Furthermore, it has been demonstrated that *Hedyotis diffusa* inhibits the growth of cancer cells by inhibiting cyclins and cyclin-dependent kinases (CDKs), thereby arresting the cell cycle at critical checkpoints. The herb also plays a crucial role in impeding metastasis by downregulating matrix metalloproteinases (MMPs), which are responsible for tumor invasion and migration. Furthermore, *Hedyotis diffusa* exerts anti-angiogenic effects by modulating VEGF expression, reducing the blood supply to tumors and restricting their growth. Its ability to enhance immune responses, especially by stimulating natural killer (NK) and T cells, further contributes to its anticancer efficacy. Additionally, the herb exhibits strong anti-inflammatory and antioxidant properties, mitigating oxidative stress and chronic inflammation, which are key factors in tumor progression. These multifaceted mechanisms

suggest that *Hedyotis diffusa* could serve as a potential complementary or alternative therapeutic agent in cancer treatment.

Anti-Proliferative Effects

Hedyotis diffusa exhibits multifaceted mechanisms in cancer therapy, targeting various aspects of tumor development and progression. It demonstrates antiproliferative effects by inhibiting cancer cell growth and survival, often through cell cycle arrest. The herb induces apoptosis via both mitochondrial and death receptor pathways, activating key caspases and pro-apoptotic proteins. Its anti-metastatic properties suppress cancer cell invasion and migration, while its anti-inflammatory effects modulate the tumor microenvironment, reducing inflammation-driven cancer progression. Additionally, *Hedyotis diffusa* boosts immune responses against cancer cells and inhibits angiogenesis, which stops the development of new blood vessels necessary for the growth and dissemination of tumours. These interrelated processes highlight its potential as a whole anticancer treatment. The study highlights the multifaceted anticancer effects of *Hedyotis diffusa Willd (HDW)*, particularly its role in inhibiting the expansion of colon cancer HT-29 cells. Using ethanol extracts of *HDW (EEHDW)*, the research demonstrates that its antiproliferative activity is mediated through cell cycle arrest at the G1/S transition, a critical checkpoint in DNA synthesis and replication. The study provides evidence that *EEHDW* downregulates key regulators of the G1/S phase, including Cyclin D1, CDK4, and PCNA, while upregulating the expression of p21, a known proliferation inhibitor. These findings underline the ability of *HDW* to disrupt uncontrolled cell division, a hallmark of cancer progression.⁴⁷ The chloroform extract of *HDW, CEHDW*, inhibits the RAS/ERK and PI3K/AKT signalling pathways to provide anticancer effects. In SW620 CRC cells, it inhibits AKT and ERK activation,⁴⁸ and downregulates Bcl-2, cyclin D1, CDK4, survivin, PCNA, and Bax, which lowers proliferation and causes apoptosis. Multiple drug resistance (MDR) is one of the main causes of chemotherapy failure. In HCT-8/5-FU cells, the ethanol extract of *HDW (EEHDW)* inhibits P-glycoprotein (P-gp), ABCG2, and PI3K/AKT pathway phosphorylation to fight drug resistance. It inhibits CDK4, PI3K, p-Akt, Bcl-2, and cyclin

D1, while upregulating Bax, p21, and PTEN, reducing cancer cell viability, colony formation, and reversing multidrug resistance (MDR).⁴⁹

Apoptosis Induction

Pro-apoptotic proteins Bax, caspase-9, and caspase-3 were activated by *EEHDW* therapy, whereas phosphorylation of the STAT3 pathway caused by IL-6 was markedly reduced. Additionally, it downregulated CDK4, cyclin D1, and Bcl-2, contributing to an increased local inflammatory milieu and accelerated tumor growth.⁵⁰ In order to combat tumour angiogenesis, *EEHDW* may also suppress VEGF-A expression levels and stop HT-29 cells from progressing from G1 to S. In a CMX model, *EEHDW* lowers intra-tumor microvascular density (MVD) early on by inhibiting target genes of the Sonic Hedgehog (SHH) signalling pathway include VEGF-A and VEGFR2. Treatment with *EEHDW* may cause DNA damage, lower mitochondrial membrane potential, and raise the HT-29 cell's Bax/Bcl-2 ratio, indicating that *EEHDW* suppressed HT-29 cell growth through a mitochondrion dependent mechanism.^{51,52} Cytochrome c, encoded by the nuclear genome, is found in the mitochondria, which regulate cell death by controlling members of the Bcl-2 family, it causes cell death.

In short, cytochrome C may be stimulated to leave the mitochondria and enter the cytoplasm by a variety of apoptosis inducers, including growth factor deficiency or DNA damage agents which then form the apoptotic complex by binding to the activating factor 1 of apoptotic peptidase (Apaf-1). This compound's synthesis initiates the caspase cascade.⁵³ Morphologically, it causes cell lysis, chromatin condensation, nuclear fragmentation, and chromosomal DNA fragmentation, blebbing, and shrinkage.⁵⁴

Anti-Metastatic Properties

The process by which epithelial cells undergo morphological and mesenchymal changes is known as the epithelial mesenchymal transition (EMT). Mesenchymal stem cells have the ability to migrate and invade, whereas the polarity and cell-cell adhesion of epithelial cells are lost. In particular, the epithelial sign E-cadherin is expressed less frequently in EMT cells, while the EMT marker Vimentin is expressed more frequently.⁵⁵ Epithelial cells contain the Ca²⁺-dependent adhesion molecule E-cadherin that is

necessary for cell differentiation and tissue growth. It is a transmembrane glycoprotein that preserves the polarity and structural integrity of epithelial cells while mediating intercellular adhesion.⁵⁶ Utilising experiments on cell colony development and wound healing, Oleanolic acid inhibited tumor cell EMT by downregulating vimentin and FOXA1 while increasing E-cadherin.⁵⁷ *EEHDW* halted the progression of the cell cycle from G1 to S by upregulating p21 and downregulating cyclin D1, PCNA, and CDK4, with effects positively related to both concentration and treatment duration.⁵⁸ By decreasing the proportion of SP in HT-29 cells and the expression of Lgr5, PCNA, ABCB1, survivin, β -catenin, and c-Myc, which are associated with the inhibition of the Wnt/ β -catenin pathway and ABC transporters,⁵⁹ *EEHDW* also inhibited CRC stem cells. Furthermore, via altering the TGF- β signalling pathway and reducing cell adhesion, migration, and invasion,⁶⁰ *EEHDW* stopped the spread of HCT-8/5-FU cells. With supplementary effects on TGF- β , Wnt/ β -catenin, SHH, ABC, and mitochondrion-dependent pathways, HDW extracts mainly target the cell cycle, PI3K/AKT, RAS/ERK, and STAT3 pathways in colorectal cancer. By encouraging apoptosis and inhibiting angiogenesis, migration, invasion, proliferation, and resistance to treatment, HDW prevents colon cancer. AKT, PIK, TP53, BRAF, CDK2, and RAF are among the important targets identified by network pharmacology. The function of HDW in controlling contacts, cell motility, and tumor-related pathways is highlighted by GO analysis.⁶¹ Through different apoptotic pathways, the H-EtOAc fraction and its main constituent, DMQ, demonstrated strong anticancer effects on HepG2 cells, according to the study. The H-EtOAc fraction engaged the death receptor pathway, whereas DMQ specifically triggered the mitochondrial apoptotic pathway. Both upregulated caspase-3, -8, and -9 activity, along with pro-apoptotic p53, influencing apoptosis and cell cycle arrest via Fas, Bax, Bcl-2, and p21. PFT- α inhibited p53-dependent apoptosis, highlighting the importance of functional p53 signaling. Given TP53 mutations' role in therapy resistance, To assess the effect of these alterations on *HDW*'s anticancer activity, more investigation is required.⁶²

Anti-Inflammatory Effects

Uncontrolled inflammation is highly

associated with the onset of tumours, metastases, and multidrug resistance.⁶³ Tumour angiogenesis and metastasis are facilitated by inflammation, and tissue microenvironments associated with chronic inflammation can contribute to the development of cancer by causing DNA damage and gene alterations.^{63, 65} Important molecular participants in tumor-suppressive and carcinogenic processes include androgen/estrogen receptors,⁶⁶ FOXM1, NF- κ B, STAT3, Wnt/ β -catenin, HIF-1 α , and NRF2. Active *HDW* ingredients like kaempferol reduce inflammation by downregulating factors such as COX-2, iNOS, IKK, and blocking the PI3K/Akt, MAPK, and VEGF pathways in RAW264.7 cells.⁶⁷ HMA lowers inflammatory cytokine levels in A549 cells by 87–81%, 34.94%, 63.64%, 72.57%, and 21.99%.⁶⁸ Additionally, quercetin (10 μ M) activates the RIG-I promoter, increasing the synthesis of IFN- α and IFN- β in human melanoma B16 and A375 cell lines.⁶⁹

Immune Modulation

The immune system's bioactivities and functions are demonstrated by the polysaccharides extracted from *HDW*. Polysaccharides can boost CD3⁺ and Cytokine-induced killer (CIK) cells, or CD56⁺, which improves CIK cells' cytolytic activity against cancer cells *in vitro*, according to earlier studies. Polysaccharide-treated CIK cells show increased IFN- α and TNF- α levels and exhibit greater cancer cell-killing efficiency compared to non-treated CIK cells.⁷⁰ By increasing the synthesis and release of inflammatory cytokines in immune cells, *HDW* extracts were also shown to have anticancer activity. This suggests that such antitumor activity should be further explored for new cancer immunotherapy.

Angiogenesis Inhibition

Angiogenesis is the process by which existing blood arteries give rise to new ones.

An essential process for the development and dissemination of cancer cells is tumour angiogenesis, therefore, inhibiting tumour angiogenesis may help prevent the progression of cancer.⁷¹ Isoscutellarein, quercetin, and kaempferol that are separated from *HDW* prevent the growth and angiogenesis of non-small cell lung cancer by inhibiting the PI3K/Akt, MAPK, and VEGF pathways.⁷² Additionally, 4-vinylphenol, a styrene metabolite from p-coumaric acid, showed hepatotoxic and pulmonary toxicity at high

doses.⁷³ In breast cancer, 4-vinylphenol (2 mg/kg) inhibited angiogenesis by suppressing the PI3K/Akt and p38 MAPK pathways, reducing fibronectin, laminin, collagen production, and VEGFR expression in HUVECs, thus decreasing endothelial cell adhesion and ECM breakdown.

RESULTS

Hedyotis diffusa (Bai Hua She She Cao) as a Therapeutic Agent in Cancer Treatment

Because of its extensive biological and pharmacological effects on cancer, *H. diffusa* has been utilised for a long time in China. This review also examines its pharmacological actions across various cancer cell lines.⁷⁴

Anti-Colorectal Cancer Activity

A common cancer, colon cancer is characterised by unchecked cell proliferation in the colon or rectum and is frequently caused by environmental, nutritional, and hereditary causes. Despite improvements in screening and treatment, it continues to be a major global cause of cancer-related death. By encouraging apoptosis, preventing cell division, and lowering angiogenesis, *Hedyotis diffusa* exhibits notable anti-colon cancer properties. Its bioactive chemicals inhibit tumour growth and metastasis by targeting pathways such as NF- κ B and Wnt/ β -catenin. *H. diffusa* is widely used in colorectal cancer (CRC) treatment. *In vitro*, its ethanol extract suppresses HT-29 cell proliferation and induces apoptosis by triggering DNA fragmentation, mitochondrial dysfunction, caspase activation, and an increased Bax/Bcl-2 ratio. It downregulates cyclin D1, CDK4, Bcl-2, and PCNA expression, inhibits G1-S progression, and upregulates pro-apoptotic Bax. These findings suggested that *H. diffusa* may have anti-colorectal cancer cell effects through a variety of mechanisms, including cell cycle arrest, the IL-6/STAT3 pathway, and the mitochondria-dependent route.⁷⁵ Animal studies also validated the mechanism.⁷⁶ At concentrations of 0.06 mg/mL to 0.20 mg/mL, the ethanolic extract of *Hedyotis diffusa* inhibited CT-26 cells in a concentration-dependent manner, with inhibition rates ranging from 35.46% to 71.84%.⁷⁷ The ethanolic extract treatment abolished 5-fluorouracil resistance in HCT-8/5-FU cells by downregulating the expression of P-gp and ABCG2.⁷⁸

Ursolic acid (IC50 71 μM) and 2-hydroxymethyl-1-hydroxy anthraquinone (IC50 45 μM), two substances that were separated from *H. diffusa*, prevented the growth of Caco-2 cells. Ursolic acid may cleave the Poly (ADP-ribose) polymerase (PARP) in order to exert its inhibitory effect.⁷⁹ Without causing any discernible damage, the ethanol extract of *HDW (EEHDW)* inhibits the growth of cancer in vivo. *EEHDW* prevented STAT3 phosphorylation in CRC mice, which reduced cell division and caused cell death. Additionally, *EEHDW* decreased CDK4, Bcl-2, and Cyclin D1 levels, while increasing p21 and Bax expression.⁸⁰

Anti-Breast Cancer Activity

Breast cancer is the most prevalent disease among women globally, which develops when breast cells grow abnormally. Because of its aggressive subtypes and metastatic potential, it continues to be a major cause of cancer-related deaths despite advancements in early identification and targeted therapy.^{81,82} *Hedyotis diffusa* has shown

potential in breast cancer treatment by inducing apoptosis, inhibiting proliferation, and blocking metastasis. Its active compounds modulate key signaling pathways, including those related to inflammation and oxidative stress, reducing tumor progression. At half maximum effective dosages (EC50) of 18.62 ± 2.71 μM for 24 hours and 42.19 ± 3.84 μM for 48 hours, methylanthraquinone produced from *H. Diffusa* inhibited MCF7 cells, causing apoptosis through the Ca²⁺/calpain/caspase-4 pathway.⁸³ Furthermore, Bcap37 cell growth and the protein tyrosine kinases v-src and pp60src were suppressed by 2-hydroxy-3-methyl anthraquinone (IC50 57 μM) and 2-hydroxy-1-methoxy anthraquinone (IC50 65 μM).⁸⁴

Anti-Liver Cancer Activity

The fifth most prevalent type of cancer is liver cancer. Hepatocellular carcinoma (HCC), the sixth most common type of cancer and the cause of almost one million fatalities each year, continues to be a significant obstacle in biomedical research. A gene mutation model serves as the

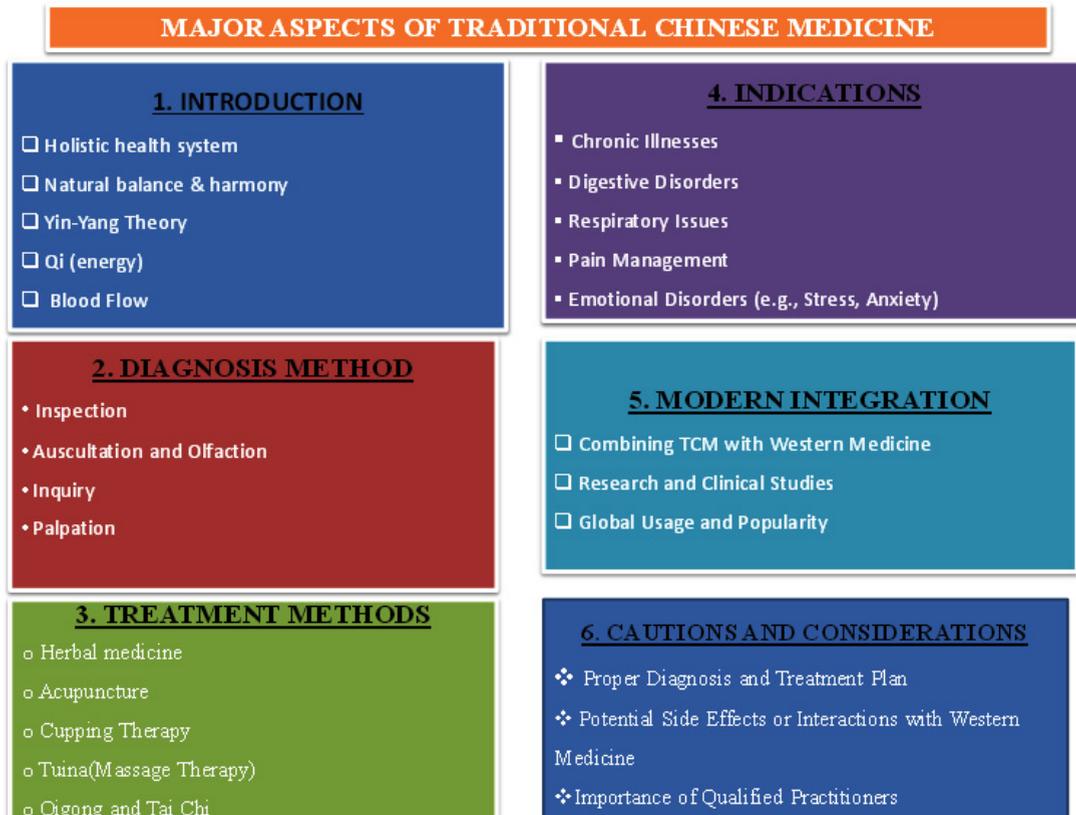


Fig. 1. Methods involved in Traditional Chinese Medicine

foundation for much of the research and treatment development techniques for cancers and cancer-related disorders.⁸⁵*Hedyotis diffusa*, a traditional medicinal herb, exhibits anti-cancer effects on liver cancer by inducing apoptosis, inhibiting tumor growth, and suppressing metastasis. Its bioactive compounds, such as iridoids and flavonoids, target cancer cell proliferation and inflammatory pathways, the prevention of blood metastases in H22 mice by an aqueous preparation of *H. diffusa*.⁸⁶After being given three doses of *H. diffusa* extract (0.25, 0.5, and 1.0 mg/kg), the weights of the body and immunological organs increased. In vitro, HepG2 cell proliferation was markedly inhibited by the aqueous extract of *H. diffusa* (1.25–10 mg/mL) through the induction of S phase delay and dose-dependent cell arrest at the G0/G1 phase.⁸⁷Additionally, the invasion of MHCC97-H

cells during the epithelial-mesenchymal transition induced by TGF- α 1 was prevented by a 200 μ g/mL dosage of *H. diffusa* complete flavone extract. Vimentin protein expression may be upregulated and e-cadherin protein expression may be downregulated in order to accomplish this.⁸⁸HepG2 cells were subjected to apoptosis by 1,3-Dihydroxy-2-Methylantraquinone (79 and 157 μ mol/L) and ethyl acetate extract (100 and 200 μ g/mL). This resulted in increased activation of caspase-3, -8, and -9 as well as dose-dependent increases in Bax, p53, Fas, FasL, p21, and cytoplasmic cytochrome C.⁸⁹Nine compounds from *H. diffusa*, including ursolic acid, oleanolic acid, and several anthraquinones, were examined for their anti-liver cancer activities at doses ranging from 1 to 200 μ M. HepG2 cell survival was considerably decreased by ursolic acid

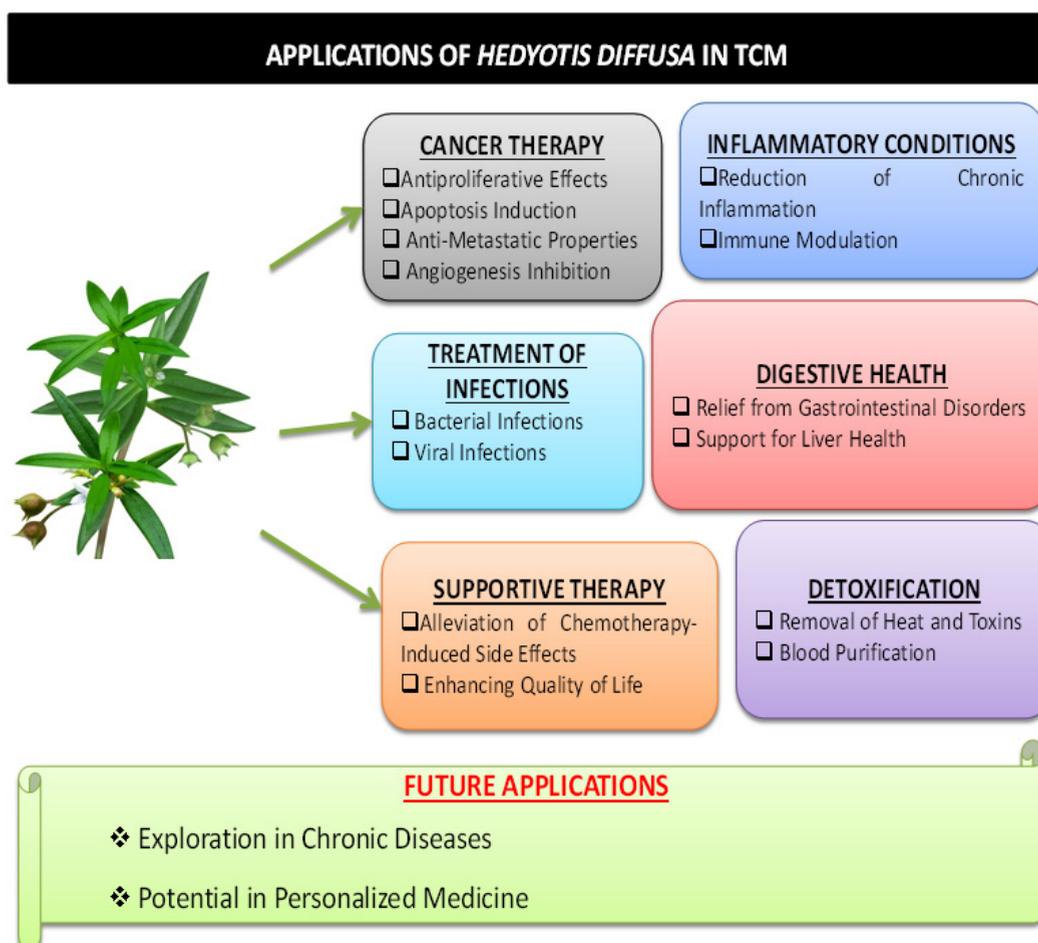


Fig. 2. Role of *Hedyotis diffusa* in Traditional Chinese Medicine (TCM)

(IC₅₀ 36.63 μ M).⁹⁰ Furthermore, the v-src and pp60src protein tyrosine kinases were inhibited by 2-hydroxy-3-methyl anthraquinone (IC₅₀ 51 μ M) and 2-hydroxy-1-methyl anthraquinone (IC₅₀ 62 μ M).⁹¹

Anti-Lung Cancer Activity

Cancerous cells can develop in the lungs, a condition known as lung carcinoma. One of the most deadly and challenging malignancies to cure, lung cancer often spreads to other parts of the body at an early stage. The World Health Organisation reports that for several decades, lung cancer has topped all cancers globally in terms of incidence.⁹² *Hedyotisdiffusa* exhibits anti-lung cancer properties by inducing apoptosis, inhibiting tumor cell proliferation, and suppressing metastasis. Its bioactive compounds regulate signaling pathways like PI3K/Akt and MAPK, reducing cancer progression. A549 and H1355 cells were shown to be concentration-dependently inhibited by treatment with an aqueous extract of

H. diffusa (0–200 μ g/mL), while LLC cells were unaffected.⁹³ Furthermore, two compounds associated with the mitochondrial apoptotic pathway, 2-hydroxy-3-methyl anthraquinone (IC₅₀ 66 μ M) and 2-hydroxy-1-methoxy anthraquinone (IC₅₀ 79 μ M), caused SPC-1-A cells to undergo apoptosis.⁹⁴ *Hedyotisdiffusa* injection significantly reduced the lifespan of lung adenocarcinoma cells in vitro and inhibited tumour growth in BALB/c nude mice in vivo. By inhibiting the Bcl-2 gene and controlling VDAC 2/3 through Bax, it caused ferroptosis. Furthermore, the study showed significant improvements in clinical symptoms, such as headache, dry mouth, sputum production, cough, chest tightness, wheezing, and shortness of breath, which ultimately improved quality of life.⁹⁵

Anti-Leukemia Activity

Hedyotisdiffusa exhibits anti-leukemia activity by inducing apoptosis, inhibiting cell proliferation, and modulating immune responses. Its bioactive compounds target key pathways such

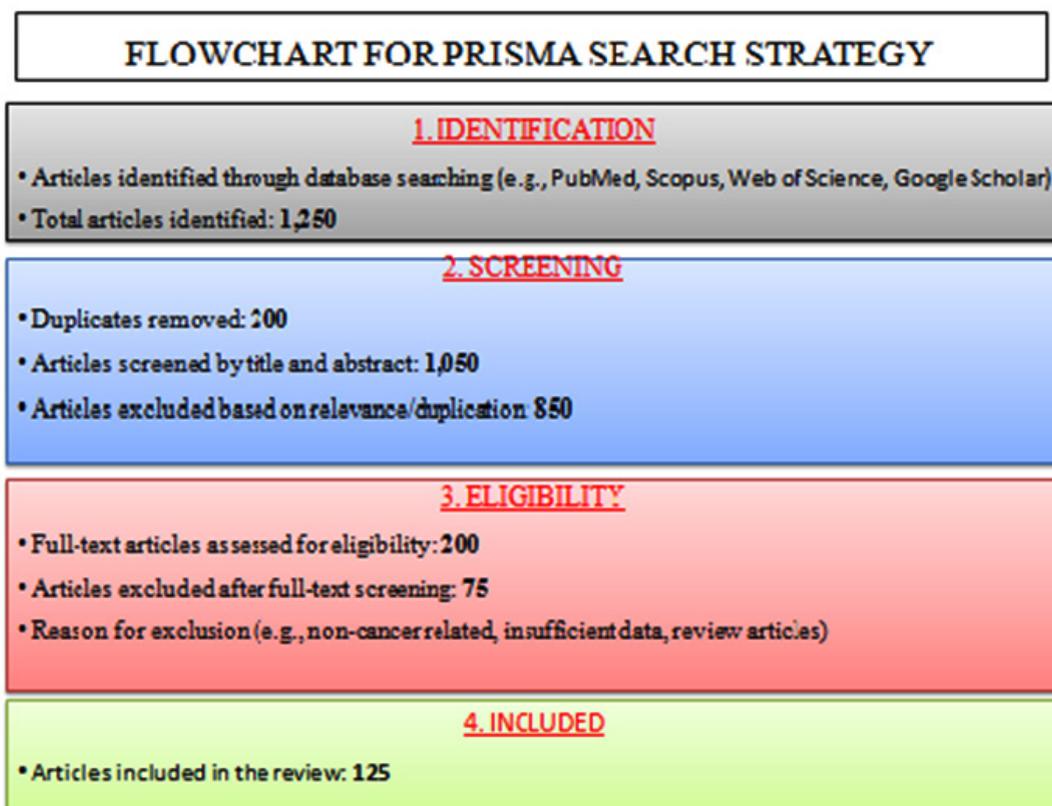


Fig. 3. Flowchart based on the PRISMA guidelines, which is typically used for systematic reviews

as PI3K/Akt and MAPK, effectively reducing leukemia progression. *H. diffusa* aqueous and ethanolic extracts have shown anti-leukemia activity in various cancer cell lines. The aqueous extract (0.01–4150 $\mu\text{g/mL}$) affected WEHI-3 cells in murine leukemia, increased T- and B-cell proliferation in leukemic animals (16 and 32 mg/kg *in vivo*), and inhibited CEM cell growth by enhancing P53 expression *in vitro*.^{96,97} The ethanolic extract (IC₅₀ 4.62 mg/mL) arrested HL-60 cells at the G₀/G₁ phase and sub-G₁ population, inducing DNA damage and activating caspase-3, -8, and -9 to inhibit cell proliferation.⁹⁸ 2-hydroxy-3-methyl anthraquinone (0–80 μM) accelerated U937 cell

death by downregulating p-ERK1/2 and activating p-p38MAPK.⁹⁹ Another study suggested it altered Fas/FasL expression and activated caspase-8 to induce apoptosis in THP-1 cells.¹⁰⁰

Anti-Cervical Tumor Activity

Its anti-cancer qualities have been emphasised by studies, especially in preventing cervical cancer cells from growing and proliferating. In cervical cancer cell lines, the plant's aqueous and ethanolic extracts have been shown to trigger apoptosis and inhibit cell cycle progression. The plant's capacity to alter important biochemical pathways involved in cell survival, proliferation, and death is thought to

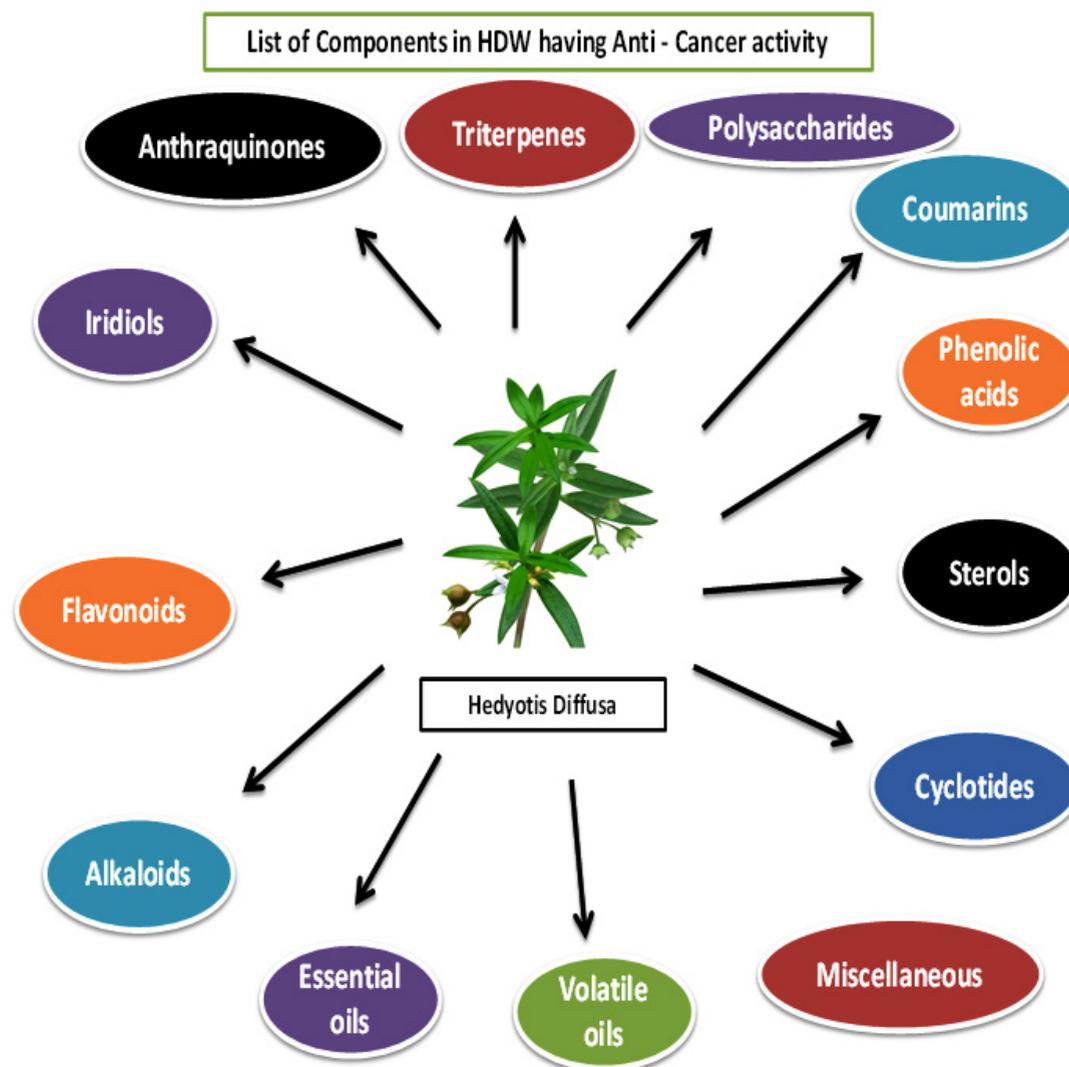


Fig. 4. Phytochemical constituents present in *Hedyotis Diffusa* plant

be responsible for these effects. Furthermore, it has been observed that *Hedyotis diffusa* increases the expression of tumor-suppressor proteins such as P53, which are essential for controlling malignant cell death. Its anti-inflammatory, antioxidant, and anti-proliferative activities further contribute to its therapeutic potential, making it a promising candidate for complementary or alternative treatment strategies in cervical cancer management. When given intragastrically to a human cervical carcinoma xenograft in naked mice, the aqueous extract of *H. diffusa* (0.5 g/kg bw) suppressed cervical cancer cells and caused HeLa cells to undergo apoptosis.¹⁰¹ With an IC₅₀ of 45 μ M,⁹⁰ anthraquinones, in particular 2-hydroxymethyl-1-hydroxy anthraquinone, demonstrated strong suppression of HeLa cells in vitro.

Anti-Prostate Cancer Activity

Several cell lines have shown that *H. diffusa*'s active chemicals have anti-prostate cancer properties. DU145, PC-3, and LNCaP cells were

inhibited by 2-methyl-3-methoxy anthraquinone (IC₅₀ 64.72–105.90 μ M), 2-hydroxy-3-methyl anthraquinone (IC₅₀ 28.82–159.20 μ M), and ursolic acid (IC₅₀ 22.33–36.08 μ M). Furthermore, 10(S)-hydroxypheophytin and 10-O-(E)-p-coumaroylscandoside methyl ester had dose-dependent anti-proliferative effects on PC-3 cells (0–60 μ M), whereas 10(S)-hydroxypheophytin A shown a strong anti-proliferative effect on LNCaP cells (IC₅₀ 20 μ M).¹⁰¹ Three cyclotides (DC 1-3) were found to have anti-prostate cancer characteristics; in particular, DC 3 (1 mg/kg) inhibited PC3, DU145, and LNCaP cells.¹⁰² Furthermore, in the prostate xenograft model, DC3 considerably reduced the tumor's development in both weight and size. At a dose of 1 mg/kg, it also had a significant anti-cancer effect ($p < 0.01$), reducing the tumour growth rate (weight) by 40.23%.¹⁰³

Immunomodulatory Effect

In leukemic mice with WEHI-3-generated leukaemia, the aqueous extract of *H. diffusa* (16 and

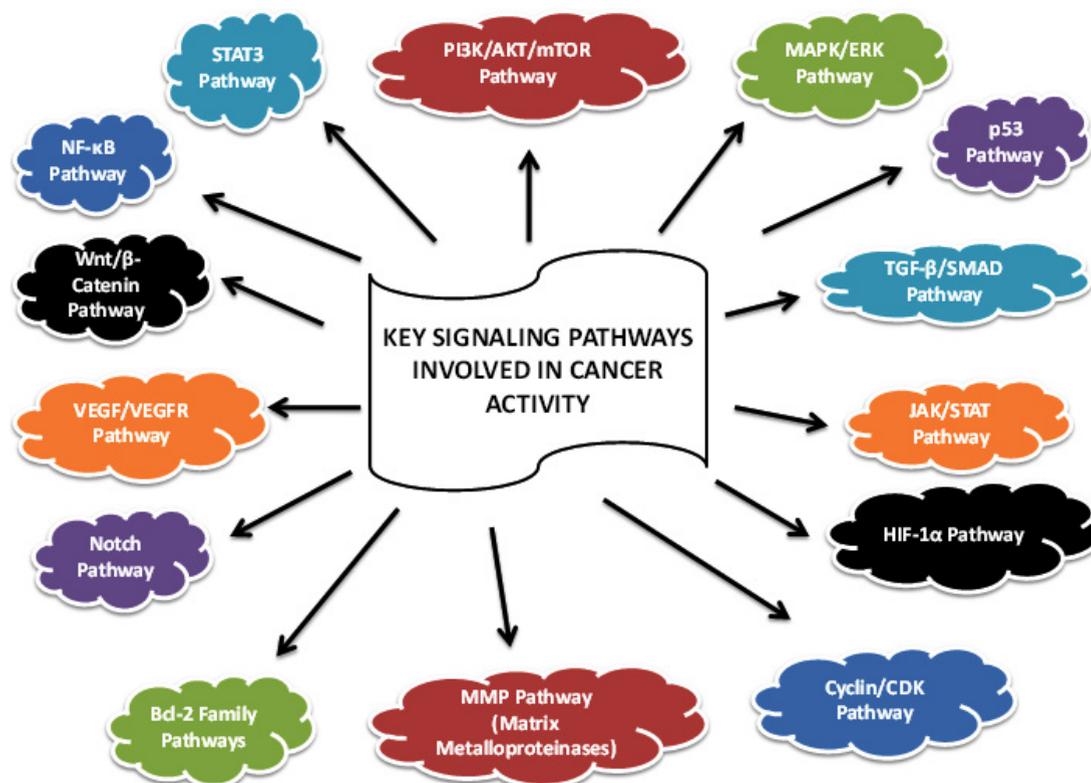


Fig. 5. Key signaling pathways involved in cancer activity

32 mg/kg) increased T- and B-cell proliferation, improving immune responses.¹⁰⁴By raising CD11b, CD19, and Mac-3 levels, improving macrophage phagocytosis, and increasing NK cell activity and B- and T-cell proliferation, the ethanolic extract (16, 32, and 64 mg/kg) also enhanced immunological responses in normal BALB/c mice.¹⁰⁵Furthermore, in immunosuppressed mice, polysaccharide extracts (2.25, 4.5, and 9.0 mg/mL) enhanced the phagocytic index, clearance index, and thymus and spleen indices.¹⁰⁶The immunomodulatory effect of total flavonoids of *H. diffusa* was demonstrated by the increased proliferation of T and B lymphocytes and the enhancement of interleukin-2 (IL-2) and interferon- α (INF- α) levels in immunosuppressed mice given oral doses of 15, 30, and 60 mg/kg.¹⁰⁷

Anti-Multiple Myeloma Activity

In RPMI 8226 cells, *H. diffusa* has been shown to have an anti-multiple myeloma impact. RPMI 8226 cell proliferation was dose-dependently reduced by injection of *H. diffusa* (20, 40, and 60 μ L/mL) 109, 2-hydroxymethyl-

1-hydroxy anthraquinone (1–200 μ M) 90, and polysaccharide extracts (1, 2, and 3 mg/mL).¹⁰⁸

Other Anti-Cancer Effects

In numerous research, *Hedyotis diffusa* has demonstrated strong anti-cancer properties. B16F10 cell growth was dose-dependently reduced by the ethanolic extract (0–200 μ g/mL).^{109,110}Its crude polysaccharide (31.25 and 62.5 mg/kg) and lipophilic extract (50 and 100 mg/kg) had anti-tumor actions on S-180 cells and provided protection against damage caused by chemotherapy.¹¹¹When *H. diffusa* was injected into MG-63 cells, the Bax gene was upregulated (50–400 μ g/mL).¹¹²Its impact was amplified when paired with cisplatin, most likely due to the regulation of Bcl-xl, Bcl-2, Bax, Bad, caspase-3, and caspase-8.¹¹⁴By triggering mitochondrial death through the AKT/ERK pathways, the aqueous extract (2–8 mg/mL) suppressed the proliferation of U87 cells.¹¹³Furthermore, human endothelial cells showed anti-angiogenic activity in response to 4-vinyl phenol from *H. diffusa* (IC50 15.31 μ g/

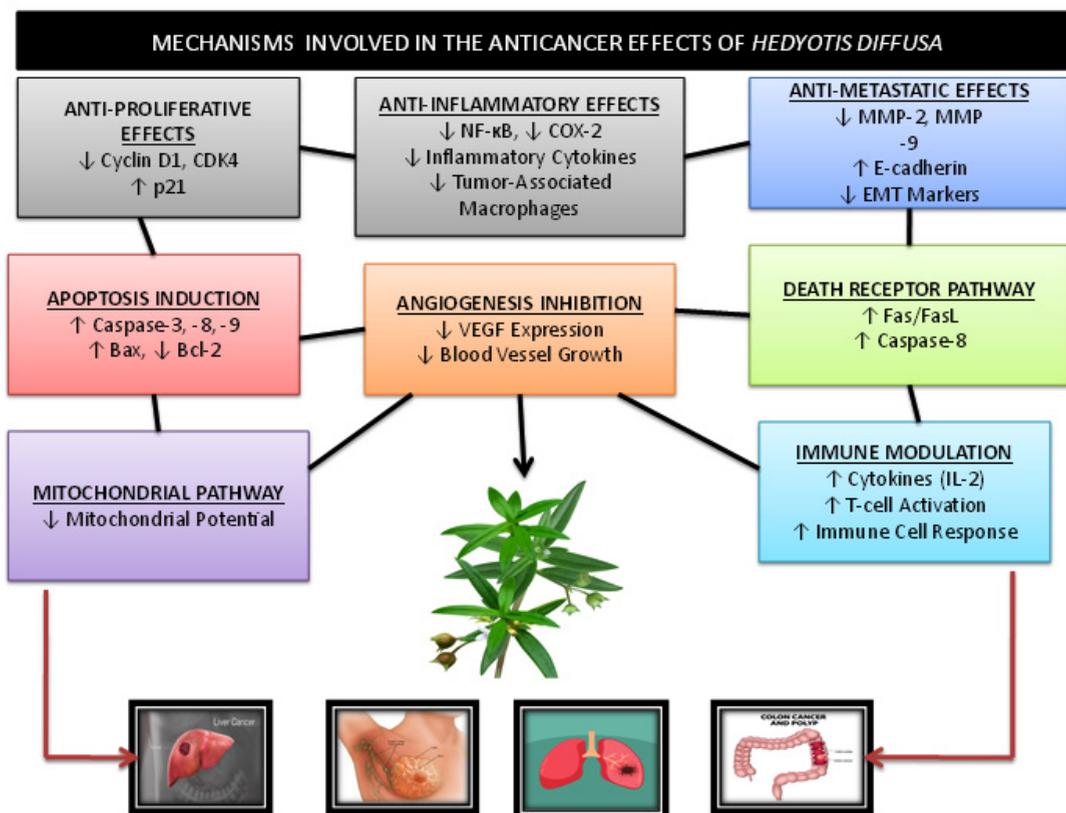


Fig. 6. Mechanisms involved in cancer prevention

mL), breast tumor-bearing mice, and zebrafish embryos, linked to the PI3K/AKT pathway. The efficacy of *Hedyotisdiffusa* in cancer therapy presents a compelling alternative or complement to conventional treatments such as chemotherapy,¹¹⁵ and radiation.¹¹⁶ Unlike these standard therapies, which often cause severe side effects like toxicity, immune suppression, and diminished quality of life, *Hedyotisdiffusa* exhibits targeted anticancer activities with lower toxicity profiles. Its bioactive compounds selectively induce apoptosis, inhibit proliferation, and suppress metastasis in cancer cells while sparing healthy tissues. Additionally, *Hedyotisdiffusa* demonstrates immune-modulatory and anti-inflammatory properties, addressing the tumor microenvironment a limitation often unmet by conventional therapies.¹¹⁷ While chemotherapy and radiation are effective in many cases, their efficacy is often diminished by drug resistance, a challenge that *Hedyotisdiffusa* mitigates through its multifaceted mechanisms. These advantages underscore its potential as a valuable addition to cancer treatment regimens, particularly in reducing side effects and enhancing therapeutic outcomes.

Synergistic Effects with Modern Cancer Therapies

Hedyotisdiffusa demonstrates significant potential for synergistic effects when combined with modern cancer therapies, enhancing efficacy and reducing side effects. For example, studies have shown that its bioactive compounds can amplify the cytotoxic effects of chemotherapy agents like cisplatin and doxorubicin while mitigating their toxicity to healthy tissues.¹¹⁸ Similarly, by making cancer cells more sensitive to radiation-induced damage, *Hedyotisdiffusa* has been shown to enhance the results of radiation therapy, possibly through mechanisms like enhanced oxidative stress and apoptosis induction.¹¹⁹ Additionally, when used alongside targeted therapies such as tyrosine kinase inhibitors, *Hedyotisdiffusa* may help overcome drug resistance by modulating pathways like NF- κ B and STAT3. By lowering side effects associated with treatment, these synergistic interactions not only increase therapeutic efficacy but also enhance patients' quality of life.¹²⁰

DISCUSSION

Traditional Chinese medicine is less

harmful to healthy cells than chemotherapy drugs. By upgrading regulatory procedures and authorising a number of herbal extracts and Chinese herbal formulations for clinical trials, such as BZL101 and PHY906,¹²¹ the US Food and Drug Administration (FDA) has promoted the use of herbal medicine in the treatment of cancer. *Hedyotisdiffusa* (*HDW*) is a popular Chinese herbal remedy; at the moment, it is a major component in 15% of the 1,700 herbal compounds used to treat cancer.¹²² According to the theories of traditional Chinese medicine, qi and blood stagnation and the buildup of poisonous heat are the causes of tumours. According to studies, *HDW* and *Scutellariabarabata* are essential for heat-clearing and detoxifying in therapeutic settings. At 5.1%, *HDW* is the most commonly used herb among the top 10 Chinese herbal formulations prescribed for patients with post-surgical colon cancer. Through in vitro investigations, contemporary research on *Hedyotisdiffusa* (*HDW*) has produced strong evidence of its anticancer properties, which are consistent with its clinical uses in traditional Chinese medicine. Among the many bioactive anticancer chemicals found in *HDW* are cyclopeptides, anthraquinones, flavonoids, iridoids, terpenes, and steroids.¹²³ Although these substances are also present in other botanical sources, *HDW*'s anticancer effects are a result of their combined existence. Its methods of action include protective autophagy, anti-inflammatory and antioxidant actions, and the inhibition of angiogenesis and cancer cell proliferation while inducing apoptosis through host immune response modulation. The main signalling pathways impacted by *HDW*'s active ingredients include the Wnt/ β -catenin, PI3K/Akt, Jak/STAT, MAPK, Bcl-2, p53, NF- κ B, TNF, and Ras-Raf pathways. By focussing on several pathways implicated in tumour growth and survival, these molecular interactions demonstrate *HDW*'s promise in cancer treatment. The investigation of traditional Chinese medicine's potential anticancer effects has drawn more attention in recent years. To clarify the underlying molecular pathways and pinpoint the exact targets that control the beginning, development, and metastasis of cancer, more research is necessary because human carcinogenesis is a complicated, multi-step process. *HDW*'s flavonoids, volatile oils, phenolic acids, and their derivatives are

mainly responsible for its anticancer properties. Although iridoids have shown strong anticancer activity *in vitro*, it is still unknown how exactly they work. Furthermore, even though the anticancer molecular mechanisms of *HDW*'s ethanol and water extracts have been thoroughly studied, more research is necessary to identify the precise active ingredients causing these effects. Future research ought to focus on elucidating their exact modes of action and pinpointing particular molecular targets for certain cancer types. The majority of *Hedyotisdiffusa* (*HDW*) formulations on the market today are injectable or oral. In order to establish a glioma-targeting system (MC-DA7R-LS), DA7R, modified with MC (MC-DA7R), was immobilised onto PEGylated liposomes.¹²⁴ This approach specifically targeted glioma cells and glioma stem cells (GSCs) and successfully penetrated the blood-brain barrier. Several studies suggest that combining multiple components or extracts from *HDW* is more effective than using a single compound, as it can modulate multiple antitumor signaling pathways simultaneously. Future research should focus on developing improved formulations, such as integrating *HDW* with different drug delivery systems to enhance targeted action on specific pathways and focus on exploring its synergistic potential with other Traditional Chinese Medicine (TCM) herbs to enhance its anticancer efficacy. Additionally, combining *HDW* with other anticancer drugs may help improve cancer cell sensitivity to chemotherapy, potentially enhancing treatment efficacy. Future research on *Hedyotisdiffusa* should Modern pharmacological approaches, such as nanotechnology and advanced drug delivery systems, could be employed to improve its bioavailability and therapeutic outcomes. Comprehensive clinical trials and rigorous safety assessments are essential to validate its efficacy and establish standardized protocols for its use in cancer treatment. Further investigations into the molecular pathways targeted by *Hedyotisdiffusa* will provide deeper insights into its mechanisms of action and enable the development of targeted therapies. Additionally, integrating *Hedyotisdiffusa* into personalized cancer treatment strategies, leveraging TCM principles alongside modern medicine, could revolutionize patient-specific therapeutic approaches.

In addition to its therapeutic effects, both short-term and long-term administration of *Hedyotisdiffusa* may present certain adverse effects. Short-term use has occasionally been associated with gastrointestinal discomfort and allergic reactions in sensitive individuals. Long-term consumption, particularly in high doses, may lead to liver or kidney function alterations, although comprehensive human data is limited. Furthermore, *Hedyotisdiffusa* may interact with conventional chemotherapeutic agents by modulating cytochrome P450 enzyme activity, potentially affecting drug metabolism and efficacy. These aspects highlight the need for cautious integration with standard cancer therapies and underscore the importance of further pharmacokinetic and toxicity studies¹²⁵.

CONCLUSION

Hedyotisdiffusa has demonstrated significant anticancer potential in Traditional Chinese Medicine (TCM), with its ability to inhibit tumor growth, induce apoptosis, suppress metastasis, and modulate the immune response. Its low toxicity and mechanisms of action position it as a promising complementary therapy to conventional cancer treatments. However, while preclinical studies highlight its efficacy, To confirm these results in clinical settings, more investigation is required. Comprehensive trials, safety assessments, and exploration of its integration into modern therapeutic protocols are essential to establish its role in mainstream oncology. Increasing our knowledge of *Hedyotisdiffusa* may lead to more individualised and comprehensive cancer treatment strategies.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not applicable.

Author's Contribution

Binoy Varghese Cheriyan: Visualization, Supervision, Project Administration; Jubilee Ramasamy :Funding Acquisition, Resources; RithikhaSooriyaprakash : Data Collection, Analysis; Sam Jebaraj : Analysis; KanigaPandi: Writing – Original Draft.

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