

Phytochemical Profiling and Anticancer Potential Of Bioactive Compounds Isolated from *Acorus Calamus*

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Abstract: *Acorus calamus* is a traditional medicinal plant rich in bioactive compounds with significant pharmacological potential. Recent research highlights its anticancer activity, primarily due to compounds such as β -asarone, α -asarone, and flavonoids. This review focuses on phytochemical profiling, isolation techniques, bioactive characterization, and anticancer evaluation of *Acorus calamus* constituents. Advanced formulation strategies such as nanoparticles and phytosomes are also discussed to enhance efficacy and bioavailability of these natural anticancer agents.

Keywords: *Acorus calamus*, β -asarone, α -asarone, bioactive compounds

I. INTRODUCTION

Cancer is a global health challenge requiring novel therapeutic approaches. Natural products continue to provide promising anticancer agents due to their chemical diversity and low toxicity. *Acorus calamus* has traditionally been used in neurological, inflammatory, and gastrointestinal disorders. Recent studies have investigated its phytochemicals for cytotoxic, antiproliferative, and apoptosis-inducing effects on various cancer cell lines.

Acorus calamus, commonly known as sweet flag, is a perennial medicinal plant widely used in traditional systems of medicine for its diverse therapeutic properties, including neuroprotective, anti-inflammatory, antioxidant, and digestive benefits (Huang, Li, & Wang, 2013). In recent years, scientific research has increasingly focused on its phytochemical constituents due to their promising pharmacological potential, particularly in cancer therapy.

The rhizomes of *Acorus calamus* are rich in bioactive compounds such as β -asarone, α -asarone, acorenone, flavonoids, and essential oils, which have been reported to exhibit significant cytotoxic, antiproliferative, and apoptosis-inducing effects on various cancer cell lines (Singh & Kaur, 2017; Choudhary & Singh, 2018). Phytochemical profiling using advanced techniques like gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC–MS) has enabled the precise identification and quantification of these bioactive constituents, facilitating their pharmacological evaluation (Verma, Mishra, & Rastogi, 2021).

Among the identified compounds, β -asarone has received particular attention for its potent anticancer activity, including induction of apoptosis, cell cycle arrest, and inhibition of tumor cell proliferation (Singh, Gupta, & Sharma, 2018). Despite its therapeutic potential, challenges such as low solubility, bioavailability, and toxicity of crude extracts necessitate the development of advanced formulation strategies, including nanoparticle-based delivery systems and phytosome complexes, to enhance efficacy and targeted delivery of the bioactive compounds (Patel & Mehta, 2020).

The integration of phytochemical profiling with in-vitro and in-vivo anticancer studies provides a comprehensive understanding of the therapeutic potential of *Acorus calamus*, paving the way for its development as a natural anticancer agent. This review aims to summarize current advances in the identification, characterization, and anticancer

evaluation of bioactive compounds from *Acorus calamus*, highlighting the potential of these natural products in cancer drug discovery.

PHYTOCHEMICAL PROFILING OF *ACORUS CALAMUS*

Phytochemical investigation involves extraction, isolation, and characterization of bioactive compounds. Techniques such as GC–MS, HPLC, and LC–MS are widely used. Major bioactive compounds include Phytochemical profiling of *Acorus calamus*, a traditional medicinal plant widely known for its therapeutic applications, has revealed a diverse range of bioactive compounds with significant anticancer potential. The rhizomes of *Acorus calamus* are rich in phenylpropanoids such as β -asarone and α -asarone, sesquiterpenes including acorenone, as well as flavonoids, tannins, and essential oils, which collectively contribute to its pharmacological properties (Singh & Kaur, 2017; Verma et al., 2021).

Advanced analytical techniques, such as gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC–MS), have been extensively employed to identify and quantify these phytochemicals with high precision (Huang, Li, & Wang, 2013). β -Asarone, the major constituent, exhibits potent cytotoxic effects on various cancer cell lines, including MCF-7 breast cancer, A549 lung cancer, and HT-29 colon cancer cells, primarily through apoptosis induction and cell cycle arrest (Singh, Gupta, & Sharma, 2018).

Similarly, α -asarone and acorenone have demonstrated antiproliferative and antioxidant-mediated cytotoxicity in vitro, suggesting their potential as chemopreventive agents (Choudhary & Singh, 2018). Flavonoids and phenolic acids from *Acorus calamus* further enhance its anticancer activity by scavenging free radicals, modulating oxidative stress, and regulating key signaling pathways involved in tumor growth (Kumar & Joshi, 2019).

The integration of phytochemical profiling with bioactivity-guided fractionation has facilitated the isolation of compounds with high therapeutic relevance, while formulation strategies, such as nanoencapsulation and phytosome systems, have improved their solubility, stability, and bioavailability for targeted anticancer applications (Verma, Mishra, & Rastogi, 2021). Collectively, these studies underscore the importance of comprehensive phytochemical profiling of *Acorus calamus* as a critical step in drug discovery and development, providing a foundation for the design of novel, plant-based anticancer therapeutics.

β -Asarone – phenylpropanoid with anticancer and antioxidant activity.

α -Asarone – phenylpropanoid inducing apoptosis in cancer cells.

Acorenone – sesquiterpene with cytotoxic effects.

Flavonoids and phenolic acids – antioxidant and antiproliferative potential.

Table 1: Major Phytochemicals in *Acorus calamus* and Their Anticancer Activities

Phytochemical	Chemical Class	Extraction Method	Reported Anticancer Activity
β -Asarone	Phenylpropanoid	Methanol, Ethanol	Cytotoxicity, apoptosis induction
α -Asarone	Phenylpropanoid	Methanol	Cell cycle arrest, antiproliferative
Acorenone	Sesquiterpene	Essential oil distillation	Cytotoxic and antiproliferative
Flavonoids	Polyphenols	Solvent extraction	Antioxidant, apoptosis induction
Essential oils	Volatile compounds	Steam distillation	Inhibition of tumor cell proliferation

ANTICANCER POTENTIAL OF *ACORUS CALAMUS* BIOACTIVE COMPOUNDS

The rhizomes of *Acorus calamus* (commonly known as sweet flag) are a rich source of bioactive phytochemicals, which have attracted significant attention due to their potential anticancer properties. Phytochemical profiling of *Acorus calamus* has revealed a diverse array of secondary metabolites, including phenylpropanoids such as β -asarone and α -asarone, sesquiterpenes like acorenone, flavonoids, and other volatile oils, which collectively contribute to its pharmacological potential (Singh & Kaur, 2017; Verma et al., 2021).

The extraction and characterization of these compounds are typically carried out using advanced analytical techniques such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS), which allow for accurate identification and quantification of bioactive constituents (Huang et al., 2013). Among these compounds, β -asarone has been extensively studied for its cytotoxic effects against a variety of human cancer cell lines, including breast (MCF-7), colon (HT-29), and lung (A549) cancer cells.

Studies have demonstrated that β -asarone induces apoptosis in cancer cells by activating caspase pathways, promoting DNA fragmentation, and modulating key signaling pathways involved in cell proliferation and survival (Singh et al., 2018). Additionally, α -asarone has shown the capacity to arrest the cell cycle at the G0/G1 phase and inhibit cancer cell proliferation, further underscoring the potential of these phytochemicals as anticancer agents (Choudhary & Singh, 2018).

Beyond apoptosis and cell cycle arrest, the antioxidant properties of flavonoids and phenolic acids present in *Acorus calamus* contribute to the overall anticancer effect by neutralizing reactive oxygen species (ROS) that can promote tumor growth (Kumar & Joshi, 2019). The essential oil fraction, containing volatile compounds, has also been reported to exhibit cytotoxicity against tumor cells, suggesting a synergistic effect among multiple phytoconstituents (Singh & Sharma, 2019).

To enhance the therapeutic potential of these compounds, formulation strategies such as nanoparticle encapsulation and phytosome complexes have been employed, which improve solubility, bioavailability, and targeted delivery to tumor tissues (Verma et al., 2021; Gupta & Kumar, 2020). In vitro and in vivo studies of these formulations have demonstrated significant improvements in anticancer efficacy, with reduced toxicity to normal cells, highlighting the promise of *Acorus calamus* bioactive compounds for translational cancer therapy.

Collectively, the phytochemical profiling of *Acorus calamus* provides a foundation for identifying potent anticancer agents, while mechanistic studies support their therapeutic relevance. Continued exploration of these bioactive compounds, coupled with innovative formulation techniques, offers a promising avenue for developing natural-product-based anticancer drugs, addressing the need for effective and less toxic alternatives to conventional chemotherapy (Patel & Mehta, 2020; Mishra & Singh, 2020).

IN-VITRO CYTOTOXICITY

β -asarone demonstrates cytotoxic effects against breast, colon, and lung cancer cell lines, reducing cell viability and inducing apoptosis (Singh et al., 2018).

MECHANISM OF ACTION

Apoptosis induction via caspase pathway

Cell cycle arrest at G0/G1 phase

Inhibition of tumor proliferation

Antioxidant-mediated cytotoxicity

FORMULATION STRATEGIES TO ENHANCE ANTICANCER EFFICACY

The anticancer potential of bioactive compounds isolated from *Acorus calamus*, such as β -asarone, α -asarone, and various flavonoids, has been well-documented in recent studies (Singh & Kaur, 2017; Verma et al., 2021). Despite their promising pharmacological activities, these compounds often exhibit poor water solubility, low bioavailability, rapid metabolism, and limited stability, which can significantly reduce their therapeutic efficacy in vivo. To overcome these limitations, advanced formulation strategies have been developed to enhance delivery, bioavailability, and targeted anticancer activity.

Nanotechnology-based delivery systems have emerged as one of the most promising approaches to improve the pharmacokinetic and pharmacodynamic properties of phytochemicals from *Acorus calamus*. For instance, β -asarone-

loaded nanoparticles have demonstrated enhanced cellular uptake in lung and breast cancer cells, resulting in improved cytotoxicity compared to crude extracts (Verma et al., 2021).

The encapsulation of bioactive compounds in biocompatible polymers such as PLGA (poly lactic-co-glycolic acid) and chitosan not only stabilizes the phytochemicals but also provides controlled and sustained release, reducing systemic toxicity and improving therapeutic outcomes (Kumar & Agarwal, 2021). Phytosome formulations represent another advanced strategy that has been successfully applied to *Acorus calamus* phytochemicals.

In a phytosome complex, the bioactive compound is bound to phospholipids, enhancing its lipophilicity and absorption across biological membranes. Studies have shown that β -asarone phytosomes exhibit higher bioavailability and improved anticancer efficacy in both in vitro and in vivo models compared to non-formulated extracts (Mishra & Singh, 2020). Such lipid-based carriers are particularly effective for compounds with poor solubility, as they facilitate passive diffusion into tumor tissues and enhance intracellular accumulation, thereby potentiating cytotoxic effects.

In addition to nanoparticles and phytosomes, nanoemulsions, liposomes, and solid lipid nanoparticles have also been explored for *Acorus calamus* bioactives. Nanoemulsions, for instance, provide a high surface area for absorption and improve the stability of volatile components such as essential oils, which are otherwise prone to degradation (Patel & Mehta, 2020).

Liposomal formulations can encapsulate both hydrophilic and lipophilic compounds from *Acorus calamus*, protect them from enzymatic degradation, and enable targeted delivery to tumor cells via the enhanced permeability and retention (EPR) effect. Furthermore, combining these formulation strategies with surface modifications, such as ligand conjugation, can allow selective targeting of tumor-specific receptors, increasing anticancer efficacy while minimizing side effects (Verma & Chaturvedi, 2022).

Overall, the integration of phytochemical profiling with advanced formulation strategies provides a robust platform to maximize the therapeutic potential of *Acorus calamus* bioactives. By improving solubility, stability, and targeted delivery, these approaches address the major limitations of crude extracts and purified compounds, enabling their development as effective anticancer agents.

Future research should focus on optimizing these formulations, conducting comprehensive pharmacokinetic and toxicity studies, and validating their efficacy in clinical models, thereby translating the promising in vitro anticancer activity of *Acorus calamus* phytochemicals into viable therapeutic options (Choudhary & Singh, 2018; Singh et al., 2018).

NANOPARTICLES

Nanoparticle-based drug delivery systems have emerged as a transformative approach in enhancing the therapeutic potential of plant-derived bioactive compounds, including those isolated from *Acorus calamus*. Traditional phytochemical extracts often face limitations such as poor solubility, low bioavailability, rapid metabolism, and non-specific distribution, which reduce their efficacy in clinical applications. Nanoparticles offer a versatile platform to overcome these challenges by enabling targeted delivery, controlled release, and improved stability of active compounds. In the case of *Acorus calamus*, bioactive constituents such as β -asarone, α -asarone, and flavonoids have demonstrated significant anticancer properties in vitro, but their clinical translation has been hindered by solubility and stability issues (Singh & Kaur, 2017; Verma et al., 2021). Encapsulation of these phytochemicals into nanoparticles enhances their bioavailability and allows for sustained release, which prolongs circulation time and increases their therapeutic index.

Recent studies have focused on synthesizing various nanoparticle systems for *Acorus calamus* bioactives, including polymeric nanoparticles, lipid-based nanoparticles, and metallic nanoparticles. For example, β -asarone-loaded polymeric nanoparticles have been formulated using biocompatible polymers such as poly(lactic-co-glycolic acid) (PLGA), which not only protect the compound from premature degradation but also facilitate controlled release at the tumor site (Verma et al., 2021). Lipid-based nanoparticles, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), have also been utilized to improve the solubility of hydrophobic compounds from *Acorus*

calamus, enabling higher intracellular uptake by cancer cells. Metallic nanoparticles, particularly silver and gold nanoparticles synthesized using *Acorus calamus* extracts, have demonstrated synergistic anticancer effects due to the combination of inherent metallic cytotoxicity and the bioactive plant compounds (Choudhary & Singh, 2018).

Nanoparticles not only improve pharmacokinetic profiles but also enhance the anticancer efficacy of *Acorus calamus* bioactives through targeted mechanisms. Encapsulated β -asarone has been shown to induce apoptosis more efficiently in breast and lung cancer cell lines compared to free compounds, through activation of caspase pathways and generation of reactive oxygen species (Singh et al., 2018). Furthermore, nanoparticle delivery systems can facilitate tumor-targeted accumulation via the enhanced permeability and retention (EPR) effect, reducing off-target toxicity and improving the safety profile of plant-based therapies. The versatility of nanoparticles also allows for surface functionalization with ligands or antibodies, further increasing specificity for cancer cells.

Overall, the integration of phytochemical profiling with nanoparticle-based delivery represents a promising strategy to harness the anticancer potential of *Acorus calamus*. By combining advanced extraction and characterization techniques with nanotechnology, researchers can develop formulations that maximize the therapeutic efficacy of bioactive compounds while minimizing systemic toxicity. Continued research is necessary to optimize nanoparticle design, evaluate long-term safety, and validate in vivo anticancer efficacy, ultimately facilitating the translation of *Acorus calamus*-derived phytochemicals into clinically relevant anticancer therapies (Patel & Mehta, 2020; Verma & Chaturvedi, 2022).

PHYTOSOME FORMULATIONS

Phytosome formulations represent a significant advancement in the delivery of plant-derived bioactive compounds, particularly those with anticancer potential such as β -asarone, α -asarone, and other phenylpropanoids isolated from *Acorus calamus*. One of the major challenges in utilizing crude plant extracts or isolated phytochemicals in cancer therapy is their limited solubility and poor bioavailability, which can substantially reduce therapeutic efficacy (Verma et al., 2021). Phytosomes are lipid-compatible molecular complexes in which the bioactive phytochemical binds to phospholipids, typically phosphatidylcholine, thereby improving its absorption across cellular membranes and enhancing systemic bioavailability (Kumar & Agarwal, 2021).

In the context of *Acorus calamus*, the primary bioactive compound, β -asarone, exhibits promising anticancer activity by inducing apoptosis, causing cell cycle arrest, and inhibiting proliferation in various cancer cell lines, including MCF-7 breast cancer and A549 lung cancer cells (Singh et al., 2018; Choudhary & Singh, 2018). However, β -asarone's hydrophobic nature limits its oral absorption and systemic circulation, which constrains its clinical applicability. Phytosome technology addresses this limitation by forming a stable complex that enhances gastrointestinal absorption and facilitates targeted delivery to tumor tissues.

Recent studies have demonstrated that β -asarone phytosomes significantly improve in-vitro and in-vivo anticancer efficacy compared to the crude extract or free compound. Verma et al. (2021) reported that β -asarone phytosomes exhibited enhanced cytotoxicity against human lung carcinoma cells and effectively inhibited tumor growth in xenograft models. The improved efficacy is attributed to increased solubility, better cellular uptake, and prolonged systemic circulation, which allow higher concentrations of the bioactive compound to reach the target site. In addition to enhanced bioavailability, phytosome formulations also offer the advantage of reduced toxicity. By complexing the bioactive compound with a lipid moiety, the phytosome minimizes nonspecific interactions with normal cells, thereby improving the therapeutic index (Kumar & Agarwal, 2021).

The preparation of *Acorus calamus* phytosomes typically involves dissolving the bioactive extract or isolated compound in a suitable solvent and reacting it with phospholipids under controlled conditions to form a molecular complex. Optimization of parameters such as phytochemical-to-phospholipid ratio, solvent type, and reaction temperature is crucial to achieving high entrapment efficiency, stability, and reproducibility (Patel & Mehta, 2020). Characterization techniques including Fourier-transform infrared spectroscopy (FTIR), differential scanning

calorimetry (DSC), and scanning electron microscopy (SEM) are employed to confirm the formation of the phytosome complex and to assess its physicochemical properties.

Overall, phytosome formulations of *Acorus calamus* bioactive compounds represent a promising strategy to overcome pharmacokinetic limitations and maximize the anticancer potential of natural products. By enhancing solubility, absorption, and targeted delivery, phytosomes facilitate more effective utilization of β -asarone, α -asarone, and other phytoconstituents, providing a potential pathway for developing novel, plant-based anticancer therapeutics. Future research should focus on large-scale production, stability studies, and clinical evaluation to translate these findings into practical cancer treatment applications (Verma et al., 2021; Singh et al., 2018; Kumar & Agarwal, 2021).

Table 2: Recent Studies on *Acorus calamus* Bioactive Compounds

Year	Study Focus	Extract Type	Cancer Model	Key Findings
2017	Phytochemical profiling	Methanol	In-vitro	Identified β -asarone, flavonoids; antioxidant activity
2018	Cytotoxic evaluation	Essential oil	MCF-7 cells	Induced apoptosis and reduced proliferation
2020	Nanoparticle formulation	β -Asarone nanoform	Lung cancer cells	Enhanced anticancer efficacy
2021	Phytosome formulation	β -Asarone phytosome	In-vitro & in-vivo	Improved bioavailability and anticancer activity

II. CONCLUSION

The phytochemical profiling of *Acorus calamus* reveals a variety of bioactive compounds with strong anticancer potential. β -Asarone, α -asarone, and other phenylpropanoids are promising candidates for anticancer drug development. Advanced formulations such as nanoparticles and phytosomes further enhance the therapeutic efficacy. Continued research is needed to explore in-vivo effectiveness and clinical translation.

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