Research Article

A Comprehensive analysis of phytochemicals in *Asparagus racemosus* extracts and their Antioxidant, Anticancer activities, and Apoptosis induction

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Abstract: Cancer remains one of the leading causes of morbidity and mortality worldwide, driven by uncontrolled cell proliferation and resistance to apoptosis. Despite significant advances in cancer treatment, including surgery, chemotherapy, and radiation therapy, challenges such as drug resistance and adverse side effects continue to limit their effectiveness. Consequently, there has been a growing interest in exploring natural compounds with potential anticancer properties. *Asparagus racemosus*, a plant known for its medicinal properties, has garnered attention for its antioxidant and anticancer effects. This study investigates the bioactive extracts of *Asparagus racemosus* for their ability to induce cytotoxicity in liver cancer cells and modulate key genes involved in apoptosis, particularly the Bcl-2 gene. Our findings reveal that the plant extracts exhibit a dose-dependent reduction in cell viability and downregulate the Bcl-2 gene expression, suggesting that *Asparagus racemosus* has the potential to serve as an alternative or complementary cancer treatment. This research contributes to the expanding body of knowledge on plant-derived anticancer agents, highlighting *Asparagus racemosus* as a promising natural therapeutic candidate for liver cancer and other malignancies.

Keywords: *Asparagus racemosus*, Bcl-2 gene, *antioxidants*, anticancer, apoptosis.

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Introduction

Cancer, a disease characterized by uncontrolled cellular growth and the potential for metastasis, remains one of the leading causes of morbidity and mortality worldwide. It encompasses over 100 distinct types, with liver cancer, particularly hepatocellular carcinoma (HCC), being a major health concern in regions such as India. Risk factors for HCC include metabolic syndrome, chronic infections with hepatitis B and C, and aflatoxin exposure, with a rising incidence observed in India [1]. Despite advancements in cancer treatment, the prognosis for many patients remains poor, especially in the advanced stages where metastasis has occurred. Chemotherapy, a cornerstone of cancer treatment, is often limited by issues such as toxicity, resistance, and side effects, further complicating its effectiveness [2]. This has led to an increasing interest in alternative therapies, which may offer less harmful and more comprehensive approaches to cancer treatment.

Research into alternative cancer therapies is crucial due to their potential to target cancer cells through unique mechanisms, reduce side effects, and

complement conventional treatments. These therapies may include herbal remedies, gene therapy, photodynamic therapy, and immunotherapy. The growing interest among patients and healthcare professionals alike in such alternatives underscores the need for rigorous, evidence-based studies to assess their safety, efficacy, and potential for integration into standard treatment regimens [3]. Additionally, exploring the mechanisms by which these therapies work, including their interactions with molecular pathways involved in cancer progression, is essential for their development into reliable treatment options.

One such promising alternative is *Asparagus racemosus*, a plant traditionally used in Ayurvedic and Siddha medicine for its diverse therapeutic properties. Studies have suggested its anticancer potential, particularly due to the presence of bioactive compounds such as steroidal saponins, alkaloids, flavonoids, and triterpenoids in its roots [4]. *Asparagus racemosus* has demonstrated anticancer activity in various cancers, including those of the liver, breast, colon, and lung, making it a candidate for further investigation [5,6]. However, the molecular mechanisms underlying its anticancer effects remain poorly understood, particularly its interaction with key apoptosis-regulating genes such as BCL2.

BCL2, an anti-apoptotic protein, plays a critical role in the regulation of cell death, and its overexpression is frequently associated with cancer cell survival and resistance to chemotherapy. Investigating the impact of *Asparagus racemosus* extract on BCL2 gene expression could provide valuable insights into its potential as an anticancer agent and its ability to modulate the apoptotic pathways in cancer cells [7]. This approach could significantly advance the field of alternative cancer therapies, offering a natural, less toxic adjunct to conventional treatments.

Furthermore, the research on natural products like *Asparagus racemosus* serves as a vital step in identifying and validating new therapeutic compounds. Natural products have historically been a rich source of novel anticancer agents, with over 40% of cancer drugs approved between 1981 and 2014 derived from plant-based sources or their derivatives [8]. This emphasizes the importance of continued exploration into plant-derived therapies, not only for their potential in treating cancer but also for their ability to provide complementary benefits when used in conjunction with conventional treatments. The integration of such complementary therapies into standard medical practice has the potential to expand the range of available treatments, improve patient outcomes, and minimize the adverse effects commonly associated with chemotherapy and other conventional therapies. Given these considerations, the aim of this study is to investigate the anticancer properties of *Asparagus racemosus* with a focus on its effects on BCL2 gene expression. Through a combination of phytochemical analysis and in vitro experiments, this research seeks to elucidate the mechanisms by which *Asparagus racemosus* may contribute to cancer treatment. By advancing our understanding of its molecular actions, we hope to provide a basis for future therapeutic

applications, furthering the development of safer, more effective alternative cancer therapies.

Methodology

Collection and Preparation of Plant Material

The roots of *Asparagus racemosus* were procured from the local market in Namakkal, Tamil Nadu, India. The collected plant material was shade-dried and ground into a fine powder. A 10 g sample of the powdered root was subjected to sequential extraction using the maceration method. The extraction process involved the use of 200 mL of ethanol followed by 200 mL of chloroform. Each extract was agitated for one week, after which it was filtered, and the solvents were removed under reduced pressure using a rotary evaporator. Once the solvent had evaporated completely, the residual extract was weighed, diluted in dimethyl sulfoxide (DMSO), and prepared for subsequent testing. The extracts were stored at a temperature range of 2–8°C for further analysis [9].

Preliminary Phytochemical Analysis

The phytochemical analysis of *Asparagus racemosus* root extracts was conducted to identify various bioactive compounds using standard methods. Alkaloids were detected through Wagner's test, which produced a reddish-brown precipitate. Carbohydrates were confirmed by Molisch's test, where a purple ring formed at the interface of sulfuric acid and alpha-naphthol. Flavonoids were identified by a yellow color change in the alkaline reagent test, while phenols were confirmed by a dark green color upon treatment with ferric chloride. Saponins were detected through persistent foam formation, and tannins were identified by a blue-green color after adding ferric chloride. Terpenoids were indicated by a reddish-brown precipitate in Salkowski's test, and quinones by a yellow precipitate upon treatment with hydrochloric acid. Steroids were confirmed by a bluish-red color change, and proteins were detected by Millon's test, which formed a red precipitate upon heating [10]. These results underscore the diverse bioactive compounds present in *Asparagus racemosus* root extracts, which may contribute to its biological effects.

Determination of Anticancer Activity of Secondary Metabolites by MTT Assay

Hepatocellular Carcinoma (Hep2) Cell Culture

Human hepatocellular carcinoma (Hep2) cells were obtained from the National Centre for Cell Science (NCCS), Pune, and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin G sodium, and 100 µg/ml streptomycin. The cells were maintained in a humidified incubator at 37°C with 5% CO2 and 95% humidity. When cells reached 80% confluence, they were harvested for sub-culture or cytotoxicity assays [11].

Cell Viability Test - MTT Bioassay

The MTT assay, based on the reduction of MTT by mitochondrial dehydrogenase enzymes in viable cells, was used to assess the cytotoxic activity of the extracts on Hep2 cells. The yellow MTT tetrazolium rings are cleaved to form purple formazan crystals, which accumulate in live cells [12].

Experiment Design

Hep2 cells at a density of 1.2 x 10^4 cells/well were seeded in 96-well microtiter plates and allowed to adhere overnight at 37°C. After discarding the medium, cells were exposed to varying concentrations (50, 100, 150, 200, 250, 300, 350, 400 µg/ml) of the plant extract dissolved in DMSO, with cyclophosphamide as the positive control and DMSO (0.2%) as the negative control [13]. Cells were incubated for 48 hours at 37°C.

Anti-Proliferation (Cytotoxicity) Assay

After 48 hours of incubation, 20 µl of MTT (5 mg/ml) was added to each well, followed by incubation for an additional 4 hours at 37°C. The medium was then discarded, and 100 µl of DMSO was added to dissolve the purple formazan crystals. The absorbance at 570 nm was measured using a microplate reader to assess cell viability [14]. The experiment was performed in triplicate. The IC50 value was determined as the concentration required to inhibit 50% of cell growth.

Cell survival and cytotoxicity were calculated using the following formulas:

Cell Viability (%) = (Test OD / Control OD) × 100

Cytotoxicity (%) = 100 – Viability (%)

Results and Discussion

Preliminary Phytochemical Analysis of *Asparagus racemosus*

Phytochemical analysis of *Asparagus racemosus* root extracts revealed the presence of several bioactive compounds. Both ethanol and chloroform extracts contained alkaloids, carbohydrates, flavonoids, phenols, sterols, terpenoids, and quinones, indicating that these compounds are soluble in both solvents. However, distinct differences were observed in the extraction of certain compounds. Tannins were exclusively present in the ethanol extract, suggesting ethanol's superior ability to extract tannins. Conversely, saponins were absent in both ethanol and chloroform extracts, indicating their low solubility in these solvents. Additionally, proteins and glycosides were found only in the ethanol extract, highlighting ethanol as a more effective solvent for these compounds. The ethanol extract exhibited a higher phytochemical yield compared to the chloroform extract (Table 1), emphasizing the importance of solvent selection in optimizing the extraction of specific phytochemicals..

Table 1. Preliminary phytochemical analysis of *Asparagus racemosus* extracts using ethanol and chloroform solvents

Phytochemicals	Types of solvent extracts	
	Ethanol	Chloroform
Alkaloids	+	+
Carbohydrates	+	+
Flavonoids	+	+
Tannins	+	-
Saponins	-	-
Phenols	+	+
Sterols	+	+
Terpenoids	+	+
Quinones	+	-
Proteins	+	-
Glycosides	+	-

Determination of Antioxidant Activity of *Asparagus racemosus*

The antioxidant activity of ethanol and chloroform extracts of *Asparagus racemosus* was assessed at varying concentrations (mg/ml), with ascorbic acid serving as the reference antioxidant. The antioxidant potential of both extracts increased with concentration. The ethanol extracts demonstrated significant antioxidant activity, ranging from 10.2% at 1 mg/ml to 67.4% at 5 mg/ml, indicating a dose-dependent increase in antioxidant potency.

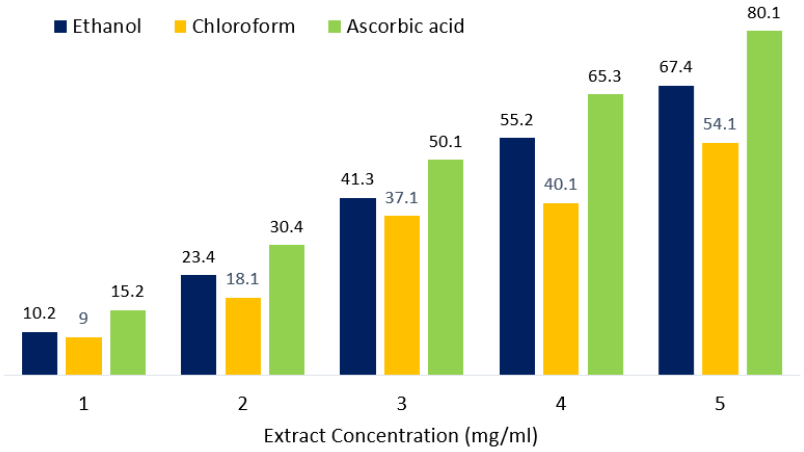

Figure 1. Antioxidant activity of ethanol and chloroform extracts at various concentrations in comparison with standard ascorbic acid.

Similarly, the chloroform extract exhibited antioxidant activity, though to a lesser extent, with values of 9.0% at 1 mg/ml and 54.1% at 5 mg/ml. Despite being less potent than the ethanol extract, the chloroform extract still displayed notable free radical scavenging activity. Ascorbic acid, the reference antioxidant, showed the highest antioxidant activity, with 15.2% at 1 mg/ml and

80.1% at 5 mg/ml. The ethanol extract had an IC50 value of approximately 3.625 mg/ml, while the chloroform extract exhibited an IC50 value of around 7.3 mg/ml (Figure 1).

Determination of Anticancer Activity of Ethanol Extracts of *Asparagus racemosus*

The anticancer activity of *Asparagus racemosus* ethanol extracts was evaluated using a cell viability assay at varying concentrations, ranging from 31.2 µg/ml to 1000 µg/ml. The results demonstrated a concentration-dependent reduction in cell viability, indicating potential cytotoxic effects of the extract on cancer cells (Table 3, Fig. 2). At the highest concentration of 1000 µg/ml, only 1.5% of cells remained viable, showing a significant reduction in cell viability. Lower concentrations, such as 125 µg/ml and 62.5 µg/ml, also led to substantial decreases in cell viability, suggesting the extract's potent ability to inhibit cancer cell proliferation..

Table 2. Cell Viability Assay of *Asparagus racemosus* ethanol extract at different concentrations

S.No	Concentration (µg/ml)	Absorbance (540nm)	Cell Viability (%)
1	1000	0.02	1.5
2	500	0.04	3.0
3	250	0.07	5.3
4	125	0.26	19.7
5	62.5	0.48	36.3
6	31.2	0.71	53.8
7	DMSO	1.32	100
8	Control Cells	1.32	100

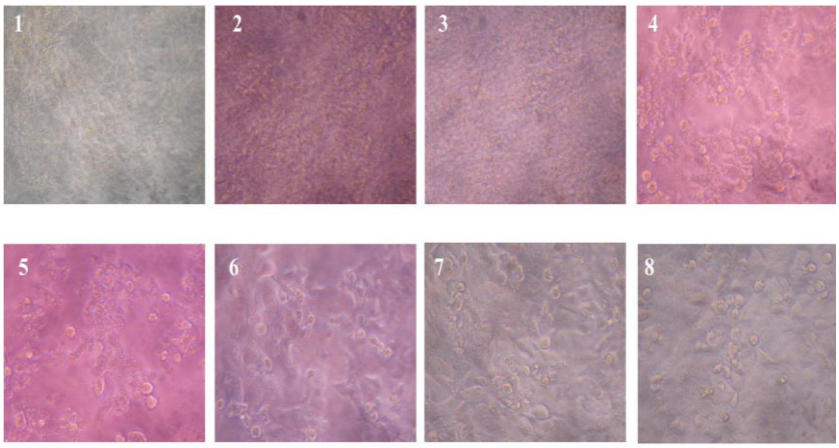

Figure 2. Effect of *Asparagus racemosus* Ethanol Extract on Hep2 Cell Viability at Different Concentrations.

In comparison to the vehicle-treated control group, which exhibited 100% cell viability, the ethanol extract demonstrated significant cytotoxicity, highlighting

its potential as an anticancer agent. The ethanol extract of *Asparagus racemosus* showed an estimated IC50 value of 42.532 µg/ml. These findings indicate that the ethanol extract possesses promising anticancer properties, warranting further investigation into its mechanisms of action and therapeutic potential in cancer treatment.

Discussion

The therapeutic potential of plant extracts in cancer treatment has garnered significant interest over the past century, with many studies investigating their anticancer properties. *Asparagus racemosus*, a plant with a long history of use in traditional medicine, has recently been examined for its biological activities, including anticancer and antioxidant effects. This study aimed to identify the phytochemicals present in *Asparagus racemosus* and assess their antioxidant and anticancer activities.

Our findings revealed that *Asparagus racemosus* contains several bioactive phytochemicals, including alkaloids, carbohydrates, flavonoids, phenols, sterols, terpenoids, quinones, proteins, and glycosides, in both ethanol and chloroform extracts. These results align with previous studies, supporting the therapeutic potential of these phytochemicals. Notably, differences in the extraction profiles of ethanol and chloroform were observed, particularly concerning glycosides, tannins, and saponins, with ethanol proving more effective in extracting proteins and glycosides. The presence of tannins and the absence of saponins in both extracts are consistent with prior studies [15,16], indicating solvent-specific solubility profiles.

The antioxidant capacity of *Asparagus racemosus* extracts demonstrated a dose-dependent increase in activity, with the ethanol extract showing superior antioxidant effects compared to chloroform extracts. This observation corroborates findings from Singh et al. (2017) [15], who reported similar dose-dependent increases in antioxidant activity. The ethanol extract's potent free radical scavenging ability suggests that *Asparagus racemosus* could be a valuable natural antioxidant source with potential health benefits.

In assessing the anticancer potential of *Asparagus racemosus* ethanol extract, a significant reduction in cell viability was observed in a concentration-dependent manner, with an IC50 value of 42.532 µg/ml. These findings are consistent with studies by Singh et al. (2018) [15] and Awati et al. (2020) [17], who also demonstrated cytotoxic effects of *Asparagus racemosus* extracts on cancer cells. Our study confirmed the anticancer properties of the plant extract, showing its ability to reduce cell viability, with the highest dose of 1000 µg/ml reducing cell viability to 1.5%. The concentration-dependent decrease in cell viability highlights the extract's potential as an anticancer agent. The findings of this study add to the growing body of evidence supporting the anticancer and antioxidant properties of *Asparagus racemosus*. The plant's bioactive compounds, particularly those extracted using ethanol, demonstrate significant therapeutic potential, particularly in the context of cancer treatment. Further

studies are needed to elucidate the molecular mechanisms underlying these effects and explore the plant's potential for therapeutic use in oncology.

Conclusion

The findings of this study underscore the significant potential of *Asparagus racemosus* as an alternative treatment for cancer, particularly in the context of liver cancer. The plant's antioxidant and anticancer properties, demonstrated through its bioactive extracts, highlight its therapeutic promise. The cytotoxic effects observed on cancer cells, coupled with the modulation of key apoptosis-related genes, emphasize the importance of exploring natural compounds in cancer therapy. Utilizing the medicinal properties of *Asparagus racemosus* may provide safer and more effective treatment options for cancer patients, offering a promising avenue for improving patient outcomes and quality of life. As a natural therapy, *Asparagus racemosus* represents a valuable addition to the evolving landscape of cancer treatment, supporting the advancement of complementary therapeutic approaches and contributing to the broader fight against cancer..

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