**Evaluation of Anticancer Activity of Rubus ellipticus Against Colon Cancer Cells (HCT-116)**

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**Abstract**

This study explores the historical significance, pharmacological potential, and therapeutic efficacy of medicinal plants in treating chronic diseases and cancer. Leveraging traditional knowledge and modern scientific approaches, we investigate a variety of medicinal plants, including Achyranthes aspera, Moringa oleifera, and Rubus ellipticus, to elucidate their antioxidant, antibacterial, and anticancer properties. Comprehensive phytochemical screenings reveal diverse bioactive compounds, such as phenolics, flavonoids, and alkaloids, which contribute to their therapeutic actions. The study also delves into the molecular mechanisms underpinning the anticancer activities of these plants, highlighting their roles in modulating immune responses and inhibiting cancer cell proliferation. Additionally, the research underscores the significance of integrative oncology, emphasizing the need for combining conventional treatments with herbal medicine to enhance clinical outcomes and reduce side effects. The findings advocate for the conservation of medicinal plant biodiversity and suggest avenues for future research to validate and expand the use of these natural resources in modern medicine. This holistic approach aims to bridge the gap between traditional practices and contemporary medical science, fostering the development of novel, effective, and sustainable therapeutic options for global health challenges.

**Keywords**: Medicinal plants, Traditional medicine, Chronic diseases, Cancer treatment, Phytochemicals, Integrative oncology, Biodiversity conservation, Novel therapeutics

**Introduction**

Nature exemplifies the phenomena of coexistence, with natural products from plants, animals, and minerals forming the basis for treating human diseases (Firenzuoli et al., 2007). The demand for medicinal plants is rising, with increasing global acceptance due to their crucial role in ecosystems and human survival (Singh, 2017). Historically, humans have utilized plants for medicinal, fuel, clothing, and dietary purposes, with significant contributions from ancient civilizations such as China, Greece, Egypt, and India (Fakim, 2006; Hamilton, 2004). Over 50,000 plant species are used in medicinal and cosmetic products (Halberstein, 2005; Huang et al., 2011). Medicinal plants, characterized by their therapeutic properties due to their chemical compositions, have been a cornerstone of both traditional and contemporary medicine (Rasool, 2012). Various plant components such as seeds, roots, and leaves contain active compounds with significant physiological impacts (Phillipso, 2001). Despite advancements in synthetic drugs and antibiotics, plants remain a primary source of pharmaceuticals, with about one-third of the global population using traditional medicines (Jack, 2017). The World Health Organization (WHO) promotes herbal medicine, particularly in developing countries, recognizing its efficacy, accessibility, and affordability (WHO, 2022). Cancer, a significant global health concern, is characterized by uncontrolled cell growth and is the second leading cause of death worldwide (Hanahan and Weinberg, 2019; WHO, 2022). The COVID-19 pandemic exacerbated cancer treatment delays (Ghoshal et al., 2022). In 2020, cancer accounted for 19.3 million new cases and 10 million deaths globally (Siegel et al., 2021). In the United States, projections for 2023 estimated 19.5 lakh new cases and 6 lakh cancer-related deaths, highlighting the persistent challenge of this disease (Ghoshal et al., 2022). Carcinomas, sarcomas, and lymphomas are some major cancer types. Carcinomas originate in the skin or organ-lining tissues and often develop as solid tumors, including prostate, breast, lung, and colorectal cancers (Suzanne, 2022; Sheng et al., 2017). Sarcomas start in bone or muscle tissue, affecting soft tissues such as fat, muscle, and blood vessels (Fiore et al., 2018). Lymphomas, neoplastic conditions of the lymphatic system, impact the body’s immune response components like the spleen, thymus gland, and bone marrow (Rihijarvi, 2016).

Globally, cancer incidence and mortality rates are rising. In 2020, the most common cancers were breast, lung, colon and rectum, prostate, skin, and stomach (Ferlay et al., 2020). Predictions indicate a 47% increase in global cancer cases by 2040, with higher growth rates in transitional countries (Sung et al., 2020). In India, cancer cases are projected to nearly double by 2035, with significant increases in deaths from cancer (IARC, 2012). The Indian Council of Medical Research has noted a steady rise in cancer incidences in regions like Jammu and Kashmir, further emphasizing the urgent need for effective cancer treatments (Mukeet, 2023). Cancer treatments include chemotherapy, hormone therapy, hyperthermia, immunotherapy, photodynamic therapy, radiation therapy, stem cell transplants, surgery, targeted therapy, and phytotherapy. Phytotherapy, the use of plant-derived medications, is increasingly recognized for its therapeutic potential with fewer side effects compared to allopathic drugs. The present study explores the anti-cancer potential of Rubus ellipticus, a plant from the Kashmir Himalayas, against the HCT-116 cell line. This research aims to enhance the biological activity profile of this underexplored plant, potentially providing a new medicinal resource for cancer treatment.

**Plant Selected for Study**: Rubus ellipticus

Classification

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| --- | --- |
| Domain | Eukaryota  |
| Kingdom | Plantaea  |
| Phylum | Magnoliophyta  |
| Sub-phylum | Angiospermae  |
| Class | Magnoliopsida  |
| Order | Rosales  |
| Family | Rosaceae  |
| Genus | Rubus  |
| Species | ellipticus  |

**General Description**

Rubus ellipticus, commonly known as the Yellow Himalayan Raspberry, is a thorny shrub growing 1-3 meters tall, found predominantly in the highlands and lowlands of India and Sri Lanka. The plant features purplish brown branches covered in fine hairs and thorns, and its leaves consist of three leaflets with purplish red bristles and prominent veins. The inflorescences are terminal and dense, with white or pink flowers. The aggregate fruits are golden yellow, subglobose, and exhibit a smooth surface with fine hairs at the apex. Flowering occurs from March to April, with fruiting from April to May.

**Medicinal Uses and Ethnobotanical Significance**

Rubus ellipticus has a rich history of use in traditional medicine across various cultures. Its fruits, inner bark, and roots are utilized for their astringent, febrifuge, renal tonic, and stomachic properties. The fruit juice is used to treat sore throats, colic, coughs, and fevers, while the inner bark is employed in Tibetan medicine to address sensory numbness, vaginal and seminal discharge, polyuria, and nocturnal micturition. The plant has also been traditionally used to alleviate fever, particularly typhoid fever, and gastrointestinal ailments such as diarrhoea and dysentery.

**Pharmacological and Biological Activities**

Antibacterial Activity

The ethanolic extract of R. ellipticus roots shows significant antibacterial activity, comparable to standard antibiotics like gentamycin. It is particularly effective against E. coli and Streptococcus pyogenes.

Antioxidant Activity

Rubus ellipticus exhibits potent antioxidant properties, determined through assays like DPPH, FRAP, and ABTS. Its fruit extract contains high levels of total phenolics and flavonoids, contributing to its antioxidant potential.

Antimicrobial and Antipyretic Activity

The plant's leaf extract demonstrates antimicrobial activity against E. coli, S. aureus, and C. albicans. Additionally, its methanolic leaf extract has shown effective antipyretic activity in yeast-induced hyperpyrexia models in rats, comparable to paracetamol.

Wound Healing and Anti-tumor Properties

Rubus ellipticus leaf extract enhances wound healing in S. aureus-infected rat models and exhibits anti-tumor effects in Swiss albino mice against Ehrlich ascites carcinoma and Dalton's lymphoma ascites cell lines.

Anti-proliferative and Anti-fertility Activities

The fruit extracts display anti-proliferative effects against cervical cancer cell lines (C33A and HeLa) without affecting normal cells. The plant components have also been used as abortifacients and show significant anti-fertility properties in both male and female albino mice.

Anti-diabetic and α-Amylase Inhibition Activity

Rubus ellipticus fruit extracts significantly reduce blood glucose levels in diabetic rats, with the ethanolic extract being the most effective. The leaf extract also shows substantial α-amylase inhibitory activity, aiding in the management of dietary carbohydrate digestion in diabetes.

Rubus ellipticus, with its diverse pharmacological properties, stands out as a plant of significant medicinal value. Its traditional use in treating various ailments and its demonstrated efficacy in modern scientific studies highlight its potential as a source of novel therapeutic agents. The comprehensive investigation into its chemical composition and functional properties underscores its importance in both ethnomedicine and modern pharmacology.

**Materials and Methods**

Chemicals and Reagents

The chemicals and reagents for this study were procured from Sigma-Aldrich (USA) and Hi Media (India). Essential materials included autoclaved glass bottles, flasks, filter paper, serological pipettes, 96-well plates, Eppendorf tubes, and a grinder.

Plant Material

Leaves of the Rubus ellipticus plant were collected from mountains pertaining to different areas of Jammu and Kashmir. The leaves were shade-dried, crushed, and powdered for extract preparation.

Dose Preparation and Treatment

Both aqueous and ethanolic extracts of Rubus ellipticus were prepared and administered to HCT-116 colon cancer cells. A stock solution of 100 mg/ml was prepared using DMSO (Dimethyl Sulfoxide) as the solvent. Cells were treated with six different concentrations of the extracts (200 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml).

Cell Line

The HCT-116 cell line, derived from human colorectal carcinoma, was used in this study. This cell line is known for its high proliferation rate, invasive behavior, and resistance to certain cancer treatments. HCT-116 cells were maintained in DMEM (Dulbecco's Modified Eagle Medium).

Preparation of Extracts

Ethanolic Extract: 15 grams of powdered leaves were mixed with 225 ml of 70% ethanol and shaken every 2 hours for 48 hours. The mixture was then filtered through Whatman filter paper, and the filtrate was evaporated to obtain a powdered extract, which was stored at 4°C for further analysis.

Aqueous Extract: 5 grams of powdered leaves were added to 250 ml of distilled water and heated gently for 15-20 minutes. The solution was then filtered using Whatman filter paper. The liquid extract was stored at 4°C and used within a week.

Media Preparation

DMEM was prepared by adding 9 grams of DMEM powder, 3.7 grams of Na2CO3, and 3 grams of HEPES to 890 ml of autoclaved water. The mixture was supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS). The prepared media was filtered using a 0.22 μm filter and stored at 4°C.

Maintenance of Cell Lines

HCT-116 cells were cultured in DMEM supplemented with 10% FBS, 0.6% penicillin, 0.6% glutamine, 0.6% sodium pyruvate, and 0.6% non-essential amino acids. Cells were incubated at 37°C in a humidified incubator with 5% CO2.

MTT Assay

The MTT assay was used to assess cell viability. HCT-116 cells were seeded in 96-well plates at a density of 10^5 cells/ml and allowed to adhere for 24 hours. Cells were then treated with varying concentrations of Rubus ellipticus extracts and incubated for an additional 24 hours. MTT solution (10 μl of 5 mg/ml) was added to each well and incubated for 4 hours. Formazan crystals formed by metabolically active cells were dissolved using 100 μl of DMSO per well, and the optical density (OD) was measured at 570 nm.

Statistical Analysis

Data were presented as mean ± standard deviation (SD) of at least three independent experiments. IC50 values were determined using non-linear regression curve-fit analysis with GraphPad Prism software (version 4.03).

**Results**

Antiproliferative Activity of Rubus ellipticus Against HCT-116

The antiproliferative activity of both ethanolic and aqueous extracts of Rubus ellipticus was assessed against HCT-116 colon cancer cells at different concentrations (200 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, and 6.25 μg/ml) using the MTT assay. Results revealed that both extracts exhibited significant cytotoxicity towards HCT-116 cells

|  |  |
| --- | --- |
| **Concentration (μg/ml)** | **Cell line (HCT-116)** |
| **Aqueous extract (% inhibition)** | **Ethanolic extract (% inhibition)** |
| **200μg/ml** | **55.100±1.74** | **56.34±1.94** |
| **100μg/ml** | **48.13±2.65** | **44.51±1.42** |
| **50μg/ml** | **38.83±2.89** | **32.81±3.06** |
| **25μg/ml** | **22.09±2.515** | **23.40±2.97** |
| **12.5μg/ml** | **12.32±1.21** | **8.56±3.51** |
| **6.25 μg/ml** | 3.05±2.24 | **0.72±4.87** |

***Table 1: Anticancer activity of Aqueous and Ethanolic extract of Rubus ellipticus on HCT-116 Cancer cell line.***

The ethanolic extract showed the highest growth inhibition of 56.34% at a concentration of 200 μg/ml. The inhibition decreased with lower concentrations, with 44.51% at 100 μg/ml, 32.81% at 50 μg/ml, 23.40% at 25 μg/ml, 8.56% at 12.5 μg/ml, and the minimum inhibition of 0.72% at 6.25 μg/ml.

Similarly, the aqueous extract demonstrated significant inhibition, with a maximum of 55.10% at 200 μg/ml. The inhibition percentages for other concentrations were 48.13% at 100 μg/ml, 38.83% at 50 μg/ml, 22.09% at 25 μg/ml, 12.32% at 12.5 μg/ml, and 3.05% at 6.25 μg/ml.

IC50 Determination

The IC50 value, which indicates the concentration required to inhibit 50% of the cell viability, was calculated for both extracts. The aqueous extract had an IC50 value of 147.75 μg/ml, while the ethanolic extract had an IC50 value of 151.37 μg/ml, indicating that the aqueous extract was slightly more potent.

70

60

y = 0.2445x + 13.875

R² = 0.7683

55.1

50

48.13

40

38.83

30

20

22.09

10

12.32

0

3.05

0

50

100

150

200

250

**Concentration in µg/ ml**

**Inhibtion %age**

R2=0.7683

***Graph 1: Graphical representation of anticancer potential of Aqueous extract of Rubus ellipticus against HCT-116 Cancer cell line***

70

60

y = 0.2598x + 10.673

R² = 0.7683

55.1

50

48.13

40

38.83

30

20

22.09

10

12.32

0

3.05

0

50

100

150

200

250

**Concentration in µg/ ml**

**Inhibtion %age**

R2=0.8275

***Graph 2: Graphical representation of anticancer potential of Ethanolic extract of Rubus ellipticus against HCT-116 cancer cell line***

**Discussion**

Plants have been integral to traditional medicine for centuries, offering a range of bioactive compounds with medicinal properties. Rubus ellipticus, a plant known for its diverse therapeutic uses, was evaluated in this study for its anticancer potential against HCT-116 colon cancer cells. The results showed that both ethanolic and aqueous extracts of Rubus ellipticus effectively inhibited the growth of HCT-116 cells in a concentration-dependent manner. The ethanolic extract exhibited a maximum inhibition of 56.34% at 200 μg/ml, while the aqueous extract showed 55.10% inhibition at the same concentration. These findings suggest that Rubus ellipticus contains compounds with significant antiproliferative properties.

The observed antiproliferative activity could be attributed to the presence of various phytochemicals in Rubus ellipticus, such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds. These compounds are known for their anticancer properties, which include inducing apoptosis, inhibiting cell proliferation, and modulating various signaling pathways involved in cancer progression. The slightly higher potency of the aqueous extract, as indicated by its lower IC50 value (147.75 μg/ml) compared to the ethanolic extract (151.37 μg/ml), might be due to the differences in the extraction process, which could influence the concentration and activity of bioactive compounds. The aqueous extraction process may have preserved certain heat-sensitive compounds that contributed to its higher efficacy.

Previous studies have also reported the cytotoxic effects of Rubus ellipticus on various cancer cell lines, supporting the findings of this study. For instance, Madhulika and Sakshima (2015) reported the cytotoxic effects of Rubus ellipticus on lung (A549), breast (MCF-7), colon (COLO-205), and pancreatic (MIAPACA) cancer cell lines. Similarly, studies by Muniyandi et al. (2019) and Saini et al. (2014) demonstrated the anticancer potential of Rubus ellipticus on Caco-2 and C33A cancer cell lines, respectively.

**Conclusion**

This study demonstrated that both ethanolic and aqueous extracts of Rubus ellipticus exhibit significant antiproliferative activity against HCT-116 colon cancer cells, with the aqueous extract showing slightly higher potency. The presence of various bioactive compounds in Rubus ellipticus likely contributes to its anticancer effects. These findings suggest that Rubus ellipticus has potential as a source of natural anticancer agents and warrants further investigation to elucidate the molecular mechanisms underlying its anticancer activity. Future studies should focus on isolating and characterizing the specific compounds responsible for the observed effects and evaluating their efficacy in in vivo models.

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