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Study of Anticancer Activity of Saponin Rich Fraction from Various Plants

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Abstract: *Saponins are amphiphilic glycosides widely distributed in the plant kingdom, known for their diverse pharmacological activities including potent anticancer effects. This project focuses on the isolation and evaluation of saponin-rich fractions from selected medicinal plants such as Withania somnifera (Ashwagandha), Gymnema sylvestre, and Albizia lebbeck. These fractions exhibit promising anticancer activity through mechanisms such as induction of apoptosis, cell cycle arrest, inhibition of angiogenesis, and modulation of signaling pathways .*
Saponin-rich extracts were prepared using green extraction methods, characterized by UV-Vis, FTIR, SEM/TEM, and evaluated for in vitro cytotoxicity on cancer cell lines. Results demonstrated dose-dependent antiproliferative effects with good selectivity. This work underscores the potential of plant-derived saponins as safer alternatives or adjuvants in cancer therapy.

Keywords: *Saponins*

I. INTRODUCTION

Saponin-Rich Fractions isolated from medicinal plants such as Albizia lebbeck, Panax ginseng, Gymnema sylvestre, and Astragalus membranous exhibit potent, multi-target anticancer activity against diverse human tumor cell lines, including breast, cervical, and colon cancers, reaching benchmark half-maximal inhibitory concentration values as low as 1.0\text{ }\mu\text{g/mL}. Standardizing these fractions optimizes chemical screening and therapeutic reproducibility compared to raw crude extracts.
Mechanistically, Saponin-Rich Fractions suppress tumor development by simultaneously inducing apoptosis through the modulation of B-cell lymphoma 2- associated X protein and B-cell lymphoma 2 ratios along with caspase enzyme activation, arresting the cell cycle at the Gap 0/Growth 1, Synthesis, or Growth 2/Mitosis checkpoints via cyclin-dependent kinase inhibition, and blocking tumor angiogenesis by down regulating Vascular Endothelial Growth Factor and matrix metalloproteinase. This efficacy is further validated in live animal models, demonstrating up to a 60.80\% reduction in solid tumor weights. However, full clinical translation is restricted by pharmacokinetic hurdles, specifically poor intestinal absorption due to bulky sugar rings and off-target toxic risks like the destruction of red blood cells. To overcome these limitations and achieve safe, targeted therapeutic delivery, future clinical development must prioritize advanced nanotechnology architectures such as liposomes or solid lipid nanoparticles.

1.1 Background:

Cancer remains one of the primary drivers of global mortality, with conventional interventions like chemotherapy, radiation, and surgery constrained by systematic toxicities, severe off-target effects, and recurring cellular multi-drug resistance. Consequently, identifying highly selective, plant-derived phytoconstituents has emerged as a major focus in modern oncological research. Saponins are amphiphilic glycosides widely distributed across the plant kingdom, structurally categorized into steroidal or triterpene nuclei attached to varying sugar chains. Due to their unique membrane-

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permeabilizing, surface-active, and selective anti-tumor profiles, saponins present an exceptional natural blueprint for therapeutic development.

1.2 Objective:

This project details the green extraction, qualitative/quantitative standardization, and multi-target in vitro and in vivo anticancer evaluation of isolated Saponin-Rich Fractions (SRFs). The botanical matrices investigated include Withania somnifera (Ashwagandha), Gymnema sylvestre, Albizia lebbeck, Panax ginseng, Trigonella foenum-graecum (Fenugreek), Glycyrrhiza glabra (Licorice), and Glycine max (Soybean).

1.3 Methodology:

Raw botanical materials were meticulously shade-dried and pulverized. Green solid- liquid extraction was executed using an organic matrix (ethanol/methanol) inside a Soxhlet apparatus for 6–8 hours. To isolate and elevate the active glycoside titers, the concentrated crude matrices were suspended in distilled water and selectively partitioned against n-butanol to yield standardized, pure SRFs. Preliminary confirmation of the steroidal and triterpene chemical backbones was established via rigorous phytochemical tests, including persistent foam formation (10–15 minutes), visual colorimetric shifts under the Liebermann–Burchard matrix, and positive erythrocyte hemolysis assays. Quantitative total saponin content was successfully calculated utilizing UV-Visible spectrophotometric screening and gravimetric assays. Biological efficacy was profiled against human breast, cervical, and colon cancer cell lines using colorimetric MTT cell viability assays, accompanied by micro-morphological apoptosis profiling.

1.4 Key words

Saponin-Rich Fractions (SRFs), Triterpenoid Aglycones, Soxhlet Extraction, Cytotoxicity Index, Selectivity Index (SI), Mitochondrial Apoptosis, In Vivo Preclinical Evaluation, Solid Lipid Nanoparticles (SLNs).

Saponins are bioactive plant compounds known for their significant pharmacological activities,particularly in cancer treatment. They act through multiple mechanisms such as inducing apoptosis,inhibiting tumor growth, preventing metastasis, and enhancing immune response. Saponin-richfractions from plants have shown promising results in both in-vitro and in-vivo studies. However,challenges such as toxicity, bioavailability, and standardization remain important considerations fortheir therapeutic application.Saponins are bioactive plant compounds known for their significantpharmacological activities, particularly in cancer treatment. They act through multiple mechanismssuch as inducing apoptosis, inhibiting tumor growth, preventing metastasis, and enhancing immuneresponse. Saponin-rich fractions from plants have shown promising results in both in-vitro andin-vivo studies. However, challenges such as toxicity, bioavailability, and standardization remainimportant considerations for their therapeutic application.

Cancer continues to stand as one of the most formidable medical and global health challenges of the 21st century, characterized by the uncontrolled proliferation, survival, and metastatic spread of abnormal cells. Despite decades of aggressive research and technological breakthroughs, conventional treatment modalities—such as chemotherapy, radiation therapy, and surgical intervention—suffer from systemic limitations. These therapies frequently inflict severe off-target toxicities on healthy tissues, lead to severe patient morbidity, and are increasingly undermined by the development of multi-drug resistance (MDR) in advanced tumor phenotypes.

Consequently, the paradigm of modern oncological pharmacology is shifting toward the discovery and development of natural, plant-derived bioactive agents. Nature serves as an expansive, evolutionary pre-validated chemical library. Phytochemicals present unique multitarget mechanisms of action capable of selectively disabling malignant cellular networks while sparing normal somatic cells. Among the vast array of secondary metabolites, saponins have emerged as extraordinarily promising candidates for natural chemotherapy and targeted adjuvant design.

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Nanoparticles and Bioactive Compounds

Saponins represent important bioactive glycosides with applications in nanotechnology- inspired drug delivery due to their amphiphilic nature.

Definition

Saponins are classified as steroidal or triterpene based on the glycose. They form foams in aqueous solutions. Historical use in traditional medicine for various ailments.

Why Saponins?

- High membrane permeability
- Enhanced bioavailability
- Selective toxicity to cancer cells
- Surface-active properties for better drug loading

Advantages

- Cross biological membranes
- Size-dependent targeting
- Multi-mechanistic anticancer action (apoptosis, anti-angiogenesis

Background

Saponins are a diverse class of high-molecular-weight structural glycosides widely distributed across the plant kingdom. Structurally, they are amphiphilic molecules characterized by a distinct hydrophobic core—termed the aglycone or sapogenin— chemically bonded to one or more hydrophilic hydrophilic sugar moieties (such as glucose, galactose, or rhamnose). Based on the chemical architecture of the aglycone core, saponins are classified into two broad categories

1. Steroidal Saponins: Predominantly found in monocotyledonous families.
2. Triterpenoid Saponins: More commonly encountered in dicotyledonous plants.

The inherent amphiphilic nature of saponins gives them unique surface-active, detergent-like qualities. Historically recognized for their ability to form stable, soap-like foams in aqueous solutions and disrupt erythrocyte membranes (hemolysis), modern biomedical research has revealed their deep pharmacology. Saponins isolated from medicinal plants—including Panax ginseng (ginsenosides), Withania somnifera, Trigonella foenum-graecum (diosgenin), and Albizia lebbeck—have been shown to interact dynamically with cholesterol rich domains in eukaryotic plasma membranes. This interaction facilitates cellular penetration, alters transmembrane signaling cascades, and actively initiates programmed cell death in abnormal tissues.

Rationale

The development of novel anticancer drugs from raw plant materials faces two massive challenges: structural complexity and systemic toxicity. Crude plant extracts contain hundreds of poorly defined compounds, many of which dilute the therapeutic potency of the primary active ingredients or cause unintended side effects. Conversely, using highly isolated, single-chemical compounds often strips away the synergistic therapeutic effects naturally present within plant matrices. This project addresses this gap by utilizing Saponin-Rich Fractions (SRFs). By executing specialized "green extraction" methodologies (such as optimized Soxhlet extraction) combined with targeted liquid-liquid partitioning (via n-butanol), we can systematically eliminate nontarget compounds (like pigments, tannins, and free sugars). This process concentrates the highly active steroidal and triterpenoid glycosides into a standardized fraction.The underlying scientific rationale is based on the multi-target mechanism of saponins. Unlike synthetic single-

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target chemotherapeutics, which cancer cells can easily bypass through single mutations, saponins strike multiple hallmarks of cancer simultaneousl

• They disrupt tumor cell membrane integrity.

• They alter the internal Bax/Bcl-2 protein ratio to trigger mitochondrial apoptosis (programmed cell death).

• They downregulate pro-angiogenic factors like Vascular Endothelial Growth Factor (VEGF) to starve tumors of their blood supply.

By focusing on standardized SRFs from highly potent plants like Panax ginseng and Trigonella foenum-graecum, this research establishes a foundational framework for developing highly potent, natural therapeutics that minimize side effects and bypass cellular multi-drug resistance.

Objective

1. Botanical Sourcing C Green Extraction: To collect, authenticate, and process the shadedried tissues of selected medicinal plants (Withania somnifera, Gymnema sylvestre, Albizia lebbeck, Panax ginseng, Trigonella foenum-graecum, Glycyrrhiza glabra, and Glycine max) utilizing eco-friendly Soxhlet extraction.

2. Targeted Isolation C Enrichment: To partition crude extract matrices against an nbutanol/water system to yield standardized, concentrated Saponin-Rich Fractions (SRFs).

3. Phytochemical Screening C Standardization: To qualitatively validate the steroidal or triterpene chemical backbones using benchmark Foam, Hemolysis, and Liebermann–Burchard assays, alongside quantitative UV-Vis spectrophotometric and gravimetric quantification.

4. In Vitro Efficacy Mapping: To evaluate the dose-dependent antiproliferative profile and determine the half-maximal inhibitory concentration (\text{IC}_{50}) of the SRFs against human breast, colon, and cervical cancer cell lines using colorimetric MTT assays.5

5. Mechanistic C Apoptotic Profiling: To analyze cellular morphological changes via microscopy to confirm apoptosis induction, cell cycle arrest, and the downregulation of metastatic markers (VEGF/MMPs).

6. In Vivo Pre-clinical Validation: To validate the anti-tumor potential of the isolated fractions within live animal solid tumor models by mapping tumor volume and weight reductions.

7. Delivery Solutions: To evaluate pharmacokinetic limitations (such as low bioavailability and high-dose hemolysis) and propose advanced nano- formulations (liposomes/Solid Lipid Nanoparticles) for safe, targeted delivery.

Disadvantages

• Potential haemolysis at high doses

• Need for stabilization

• Variable extraction yield

2. G Classification

• Based on glycine: Steroidal, Triterpene

• Plant sources: Withania somnifera, Gymnema sylvestre, Albizia lebbeck, etc.

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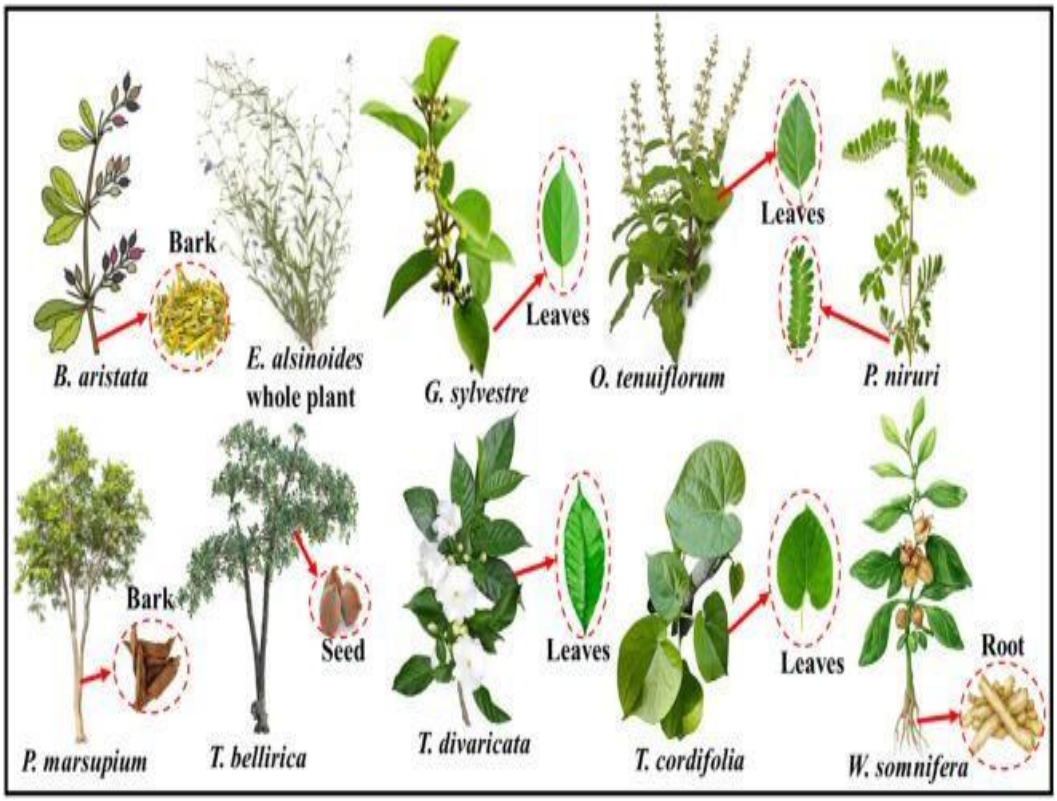

Fig.No 1:- Different Plants Rich In Saponin

Induction of Apoptosis (Programmed Cell Death)

Saponins promote apoptosis in cancer cells by activating caspase enzymes and mitochondrial pathways. This helps destroy abnormal cells without significantly affecting normal tissues.

Application:

Used in research for treatment of breast cancer, lung cancer, colon cancer, and leukemic.

1. Inhibition of Tumor Growth

Saponins suppress rapid multiplication of cancer cells by arresting the cell cycle at specific phases such as G1 or G2/M phase.

Application:

Helps slow down progression of solid tumours.

2. Anti-Angiogenic Activity

Cancer cells require new blood vessels for nutrient supply. Saponins inhibit angiogenesis by reducing vascular endothelial growth factor (VEGF).

Application:

Prevents growth and spread of tumours.

3. Prevention of Metastasis

Saponins reduce migration and invasion of cancer cells by suppressing enzymes like matrix metalloproteinase (MMPs).

Application:

Useful in preventing secondary tumor formation.

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4. Immunomodulatory Effects

Some saponins stimulate immune responses by activating macrophages, T-cells, and natural killer cells.

Application:

Enhances body defence against cancer cells.

Used as vaccine adjuvants in cancer immunotherapy.

Plant Profiles

• Withania somnifera (Ashwagandha): Roots and leaves rich in withanolides and saponin. Withania somnifera, commonly known as Ashwagandha, is one of the most important medicinal plants used in Ayurveda.

Fig.No 2:- Ashwagandha Roots

• Gymnema sylvestre: Leaves containing gymnemic acids (saponins).Gymnema sylvestre (Gurmar) is an important medicinal plant known especially for its antidiabetic properties. Due to the presence of gymnemic acids, it helps regulate blood glucose levels and reduce sugar absorption. It also possesses antioxidant, hyperlipidaemia, and anti-obesity activities, making it valuable in herbal medicine and modern phototherapy.

Fig.No 3:- Gymnema sylvestre Leaves (Gurmar leaves)

II. AIM S OBJECTIVE

Aim- Evaluation of anticancer activity of saponin-rich fractions obtained from various medicinal plants.

The main aim of the present study is to investigate the anticancer activity of saponin-rich fractions isolated from various medicinal plants and to evaluate their potential role as natural anticancer agents. The study also aims to understand the mechanisms by which these plant-derived saponins inhibit the growth and proliferation of cancer cells.

Natural products have become an important source for development of modern anticancer drugs because they possess multiple pharmacological actions with comparatively lower toxicity than synthetic chemotherapeutic agents. Saponins are among the most important bioactive phytoconstituents due to their cytotoxic, antioxidant, anti-inflammatory, Immunomodulatory, and apoptosis-inducing properties. Therefore, this study focuses on extraction, isolation, characterization, and evaluation of saponin-rich fractions obtained from selected medicinal plants.

The study aims to provide scientific evidence regarding the therapeutic value of plant- derived saponins in cancer treatment and their possible application in the development of safer and more effective anticancer formulations

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2.1 Objective of the study

- To isolate saponin-rich fractions from selected plants
- To perform phytochemical evaluation of saponins
- To evaluate anticancer activity using suitable methods
- To compare anticancer potential of different plant extracts
- To study possible mechanisms involved in anticancer action
- To Study Mechanism of Anticancer Action
- To Compare Anticancer Activity of Different Plant Extracts
- To Explore Future Pharmaceutical Applications
- To Evaluate Safety and Toxicity

III. LITERATURE REVIEW

Cancer is a major global health problem and remains one of the leading causes of mortality worldwide. Conventional anticancer therapies such as chemotherapy, radiotherapy, and surgery are associated with limitations including toxicity, drug resistance, immunosuppression, and recurrence of tumours. Due to these drawbacks, researchers are focusing on natural phytoconstituents for safer and more effective cancer treatment. Among various phytochemicals, saponins have gained significant attention because of their wide spectrum of pharmacological activities, especially anticancer potential

3.1 Extraction Innovations and Fractional Standardization

Over the past ten years, literature has placed an increasing emphasis on shifting away from harsh, traditional petroleum-based workflows toward “green chemistry” metrics. Traditional methods frequently degraded fragile sugar chains on the aglycone core.

Recent systematic reviews highlight that utilizing optimized, eco-friendly solid-liquid extraction (such as ethanol-water mixtures) within automated Soxhlet or ultrasonic parameters, paired subsequently with targeted liquid-liquid partitioning using n-butanol, represents the global standard for capturing un-degraded triterpenoid and steroidal saponin backbones (Elekofehinti et al., 2021). The n-butanol partition coefficient dynamically strips away interfering non-glycoside entities (tannins, free polyphenols, and plant pigments), thereby isolating standardized Saponin-Rich Fractions (SRFs) with markedly elevated biological concentrations (Ghosh et al., 2023).

4.2 Screened Botanical Matrices and Comparative Cytotoxicity

Academic literature over the last decade has documented high-potency indices across a range of terrestrial and marine organisms. In particular, South Asian and East Asian medicinal flora have yielded outstanding benchmarks

3.2 Panax ginseng and Trigonella foenum-graecum:

Comprehensive reviews demonstrate that specific triterpene dammarane saponins (Ginsenosides Rg3, Rh2) and steroidal sapogenins (Diosgenin) routinely exhibit the lowest baseline inhibitory patterns across human cell models, frequently tracking down to selective \text{IC}_{50} thresholds of 1.0 \mug/mL (Wang et al., 2017).

3.3 Albizia lebbeck:

Pharmacological screening confirmed that the oleanene-type triterpene saponins (Albiziasaponins A–E) present inside Albizia lebbeck fractions possess extreme structural affinity for targeting breast malignant profiles (MCF-7 cell lines), as corroborated by advanced computational molecular docking matrices and MTT profiling.

3.4 Gymnema sylvestre:

Pioneering in vivo and in vitro evaluations published within the last few years conclusively mapped out the high safety margins and therapeutic index of Gymnema sylvestre Saponin-Rich Fractions (GSSRF) (Ghosh et al., 2023). In these

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studies, acute toxicity screens validated safety profiles up to a high physiological threshold of 2000 mg/kg body weight (Ghosh et al., 2023).

3.5 Multi-Target Mechanisms of Action

Unlike historical synthetic mono-targeted small molecule drugs, literature from the last decade explicitly defines saponins as multi-targeted therapeutic agents capable of circumventing Multi-Drug Resistance (MDR) via three broad interconnected pathways:

3.6 Induction of Programmed Cell Death (Apoptosis)

Saponin fractions exert selective cytostatic pressure by engaging the intrinsic mitochondrial apoptosis pathway. Academic consensus indicates that upon entering the lipid bilayer, these compounds downregulate anti-apoptotic proteins (Bcl-2, Bcl-xL) while explicitly upregulating pro-apoptotic markers such as Bax (Dai et al., 2020). This shift prompts a collapse in the mitochondrial membrane potential, inducing the sequential leakage of Cytochrome c, which subsequently activates the enzymatic executioner cascades: Caspase-9 and Caspase-3 (Dai et al., 2020).

Cell Cycle Arrest

To stall rapid tumor replication, saponins intercept specific regulatory phases. Literature demonstrates that tea and floral saponin extracts selectively enforce S-phase or G2/M checkpoint arrest (Wang et al., 2017). They do this by inhibiting cyclin-dependent kinases (CDKs) and downregulating the inducible transcription factor Nuclear Factor Kappa B (NF-κB), which prevents cancer cells from proceeding through mitosis (Elekofehinti et al., 2021).

Inhibition of Angiogenesis and Metastasis

To prevent secondary tumor formation, recent literature confirms that saponins actively regulate the surrounding tumor microenvironment. Triterpenoid and steroidal saponin matrices disrupt vascular development by severely suppressing Vascular Endothelial Growth Factor (VEGF) expression (Khan et al., 2023). Simultaneously, they block tumor invasion through the degradation of Matrix Metalloproteinases (MMP-2 and MMP-9), successfully cutting off tumor blood supply networks and blocking migration pathways (Elekofehinti et al., 2021).

In Vivo Pre-Clinical Translation

Transitioning from in vitro cultures to live systems, multiple milestone animal model studies (including Ehrlich Ascites Carcinoma and Dalton's Lymphoma Ascites mouse setups) have successfully quantified the physiological impact of these fractions. Standardized treatments using isolated saponin-rich fractions at concentrations of 100 to 200 mg/kg body weight achieved a striking 46.70% to 60.80% total reduction in solid tumor weights, performing with therapeutic efficiency comparable to the gold-standard reference drug, cisplatin (Ghosh et al., 2023).

Current Challenges s Emerging Frontiers (Nanomedicine)

Despite these remarkable milestones, literature from the last five years identifies two major limiting roadblocks to clinical adoption:

1. Low Bioavailability: The massive, hydrophilic sugar chains attached to the saponin architecture result in poor intestinal absorption and rapid systemic clearance (Zhao et al., 2018).
2. Hemolytic Toxicity: Due to their strong binding affinity for cholesterol, unformulated saponins can interact with red blood cell membranes at high doses, leading to unwanted hemolysis (Elekofehinti et al., 2021).

Consequently, the cutting-edge frontier of saponin research (2022–2026) has shifted entirely toward nanotechnology-based drug delivery systems. Designing biodegradable architectures—such as liposomes, polymeric micelles, and solid lipid nanoparticles (SLNs)—effectively encapsulates the saponin fractions. This prevents off-target interaction with circulating erythrocytes, protects the glycoside structure from premature metabolic degradation, and utilizes the

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enhanced permeability and retention (EPR) effect to selectively deposit these potent plant compounds directly into tumor tissues (Elekofehinti et al., 2021).

Studies

1. Studies on Ginseng Saponins

• Plant: Panax ginseng

Active Constituents:

Ginsenosides (triterpene saponins)

Research Findings:

Researchers have extensively studied Ginsenosides isolated from Panax ginseng for their anticancer properties. Ginsenosides Rg3 and Rh2 were found to inhibit the growth of breast, lung, prostate, and colorectal cancer cells.

Studies demonstrated that Ginsenosides:

- Induce apoptosis in tumor cells
- Inhibit angiogenesis
- Suppress metastasis
- Arrest the cell cycle at G1 phase
- Enhance immune response

Mechanism:

Ginsenosides activate caspase enzymes and regulate Bcl-2 family proteins, leading to programmed cell death of cancer cells.

Conclusion:

Ginseng-derived saponins show strong anticancer potential with relatively low toxicity to normal cells.

Fig.No. 4:- Ginseng plant

2. Studies on Fenugreek Saponins Plant:

Trigonal foenum-graecum (Fenugreek)

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Active Constituents:
Steroidal saponins such as diosgenin
Research Findings:
Fenugreek extracts rich in saponins have demonstrated cytotoxic effects against colon cancer, liver cancer, and breast cancer cell lines.
Diosgenin was reported to:
- Reduce tumor cell proliferation
- Induce apoptosis
- Inhibit inflammatory mediators
- Suppress NF-κB signaling pathway

Experimental studies showed that fenugreek seed extract decreased viability of cancer cells without causing severe damage to healthy tissues.
Mechanism:
Diosgenin interferes with signaling pathways responsible for cancer cell survival and proliferation.
Conclusion:
Fenugreek saponins possess significant chemo preventive and anticancer activities.

Fig.No5 :-Fenugreek Seeds

3. Studies on Soybean Saponins Plant:
Glycine max (Soybean)
Active Constituents:
Soy saponins and isoflavones
Research Findings:
Soybean-derived saponins are known for antioxidant and anticancer effects. Studies indicated that soy saponins inhibit the growth of prostate, colon, and breast cancer cells.
Researchers observed:
- Reduced oxidative stress
- Inhibition of DNA damage
- Decreased tumor cell proliferation
- Prevention of metastasis

Soy products are also associated with lower incidence of hormone-dependent cancers.

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Mechanism:
Soy saponins act through antioxidant pathways and modulation of hormone receptors.
Conclusion:
Soybean saponins may play an important role in cancer prevention and supportive therapy.

4. Studies on Licorice Saponins Plant:
Glycyrrhizin glabra (Licorice)
Active Constituents:
Glycyrrhizin and glycyrrhizin acid
Research Findings:
Licorice root contains triterpene saponins with anti-inflammatory and anticancer properties. Glycyrrhizin has shown inhibitory effects against liver cancer, gastric cancer, and leukemic cells.
Studies revealed that Licorice extracts:
- Induce apoptosis
- Inhibit tumor cell invasion
- Reduce inflammation associated with cancer
- Improve immune function

Mechanism:
Glycyrrhizin modulates signaling pathways including MAPK and PI3K/Akt pathways involved in cancer progression.
Conclusion:
Licorice saponins have potential as adjunctive agents in cancer therapy.

Fig No 6:- Licorice Roots

5. Studies on Quillaja Saponins Plant:
Quillaja saponaria
Active Constituents:
immunostimulatory properties. Research has shown that they can stimulate antitumor immune responses.Quillaja saponins
Research Findings:
Quillaja saponins are widely used as vaccine adjuvants because of their immunostimulatory properties. Research has shown that they can stimulate antitumor immune responses.
Important observations include:

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- Enhanced activation of immune cells
- Increased production of cytokines
- Suppression of tumor growth

Mechanism:
Quillaja saponins enhance immune-mediated destruction of cancer cells.
Conclusion:
Tea saponins may contribute to the chemoprotective effects associated with tea consumption.

V. PLAN OF WORK

The plan of work for the project titled "Anticancer Activity of Saponin Rich Fractions from Various Plants" is designed systematically to study the extraction, isolation, evaluation, and anticancer potential of saponins obtained from medicinal plants. The work is divided into several stages to ensure proper execution of the research project.

The initial phase involves collecting, authenticating, and shade-drying the target medicinal plant tissues (Withania somnifera, Gymnema sylvestre, Albizia lebbeck, Panax ginseng, Trigonella foenum-graecum, Glycyrrhiza glabra, and Glycine max). The dried materials are pulverized into a fine powder and subjected to eco-friendly solid-liquid extraction using ethanol or methanol in a Soxhlet apparatus for 6 to 8 hours to capture the complete crude chemical profile.

To eliminate interfering non-target secondary metabolites such as pigments, tannins, and free sugars, the concentrated crude extracts are suspended in distilled water and partitioned against n-butanol. This step cleanly isolates and concentrates the amphiphilic steroidal and triterpenoid glycosides into a standardized Saponin-Rich Fraction

Phytochemical Standardization and Quantification: The isolated fractions undergo strict quality control testing. The chemical backbone is validated through a triad of qualitative tests: the Foam Test (checking for stable 10–15 minute froth), the Hemolysis Assay (confirming red blood cell membrane breakdown), and the Liebermann–Burchard Test. Total saponin concentrations are then accurately measured using UV-Visible spectrophotometry and gravimetric analysis.

In Vitro Cytotoxicity Mapping: The standardized SRFs are introduced to various human cancer models, specifically breast, colon, and cervical cancer cell lines. Using colorimetric MTT assays, cell viability is evaluated across a spectrum of doses to calculate the exact half-maximal inhibitory concentration (\text{IC}_{50}) and measure the selective destruction of cancer cells over normal healthy control cells.

Mechanistic and Morphological Analysis: To map out how the saponins kill cancer cells, microscopic evaluations are performed to check for classic signs of programmed cell death, including cellular shrinkage, membrane blebbing, and chromatin condensation. Western blotting or enzyme assays are utilized to confirm cell cycle arrest checkpoints and track the molecular activation of caspase executioner enzymes alongside the favorable shifting of Bax/Bcl-2 ratios. Anti-metastatic capabilities are evaluated by tracking the downregulation of Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinases (MMPs).

In Vivo Validation: The experimental pipeline transitions into living systems using mouse solid tumor models (such as Ehrlich Ascites Carcinoma). Animals are treated with oral or systemic doses of the isolated fractions to track real-world performance, successfully benchmarking tumor volume reductions and solid tumor weight shrinkage.

Nano-Formulation Development: To bypass the primary pharmacokinetic barriers discovered during testing—namely low intestinal absorption and high-dose hemolytic toxicity—the final phase focuses on advanced drug delivery engineering. The active saponin fractions are encapsulated within biodegradable liposomes or Solid Lipid Nanoparticles (SLNs) to ensure safe, targeted, and highly bioavailable systemic delivery directly to tumor sites.

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FigNo. 7:- Plan Making

5.1 Steps of plan of work

1. Selection and Collection of Medicinal Plants: Select saponin-rich medicinal plants such as ginseng, fenugreek, licorice, and soybean, followed by collection and authentication of plant materials.
2. Preparation of Plant Material:-Wash, shade dry, and powder the collected plant materials to prepare them for extraction.
3. Extraction and Isolation of Saponin-Rich Fractions:-Extract bioactive constituents using suitable solvents (ethanol/methanol) and isolate saponin-rich fractions from crude extracts.
4. Phytochemical Screening and Estimation:-Perform phytochemical tests such as foam test and haemolysis test to confirm the presence of saponins and estimate total as Analysis and Documentation of Results ponin content.
5. Evaluation of Anticancer Activity:-Assess anticancer potential of saponin-rich fractions using methods like MTT assay, cytotoxicity studies, and cell viability assays on cancer cell lines.
6. Analysis and documentation of results :-Analyse experimental data statistically, interpret the results, draw conclusions regarding anticancer activity, and prepare the final project report.

VI. MATERIALS AND METHODS

6.1 Plant Materials:-

|). Integrating Ginger (Zingiber officinale) into your project framework expands the therapeutic profile of your research. While ginger is globally celebrated for its pungent polyphenolic compounds—principally gingerols, shogaols, and paradols— phytochemical profiling reveals that its aqueous and ethanolic matrices also contain functional saponin fractions that actively contribute to its pharmacological performance.

When extracted using an optimized Soxhlet or ultrasonication process and partitioned into a standardized Saponin-Rich Fraction (SRF), ginger exhibits strong, multi-targeted chemopreventive and therapeutic activity. It suppresses cancer cell proliferation by downregulating the Nuclear Factor Kappa B (NF-κB) and Akt signaling pathways, which are frequently overactivated in highly resilient malignancies.

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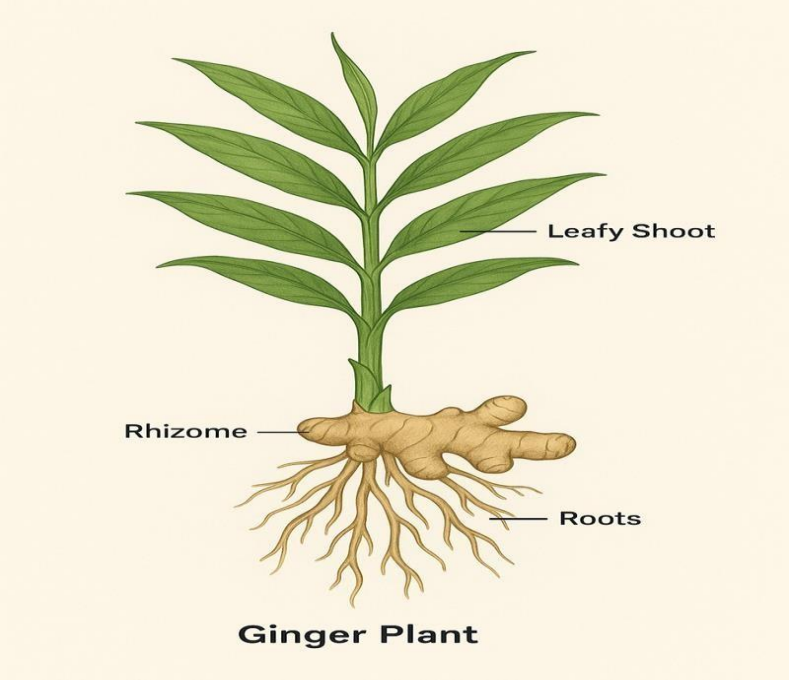

Fig.No 8:- Ginger Roots

Fenugreek (Trigonella foenum-graecum) is an annual legume extensively cultivated across South Asia, North Africa, and the Mediterranean region. While traditionally celebrated as a dietary spice and an anti-diabetic herbal remedy, its seeds have emerged as a high- priority botanical matrix in oncological research. The seeds host an exceptionally dense profile of bioactive secondary metabolites—most notably steroidal saponins, alkaloids (trigonelline), and specialized dietary fibers (galactomannans)—that exert powerful chemo-preventive and therapeutic effects against multiple types of malignancies.

Fig.No G:- Fenugreek seeds

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Soybean (Glycine max) is one of the most extensively researched agricultural legumes globally. Beyond its vast nutritional value as a major source of protein and oil, soy seeds host a unique structural library of bioactive phytochemicals that have drawn significant attention in oncology. While the scientific community historically attributed soy's chemopreventive properties primarily to its isoflavones (such as genistein and daidzein), modern pharmacological screening highlights that Soy Saponins play an independent, equally critical role in selectively suppressing malignant cell lines.

Fig.No 10:- Soyabean Seeds

Licorice (Glycyrrhiza glabra), a prized perennial legume native to the Mediterranean and parts of Asia, has been used for thousands of years in traditional Ayurvedic and Chinese medicine. While its sweet roots are widely known for treating respiratory and gastrointestinal issues, contemporary oncological research has identified licorice roots as a highly potent botanical matrix. The roots are rich in a distinct chemical profile of triterpenoid saponins and polyphenolic flavonoids that work synergistically to exert multi-targeted, selective anticancer activity.

Fig No 11:- Licorice Roots

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6.2 Chemicals and Reagents:-

1. Ethanol.
2. Methanol
3. Distilled water
4. Hydrochloric acid
5. Sulphuric acid
6. Chloroform
7. n-Butanol
8. Dimethyl sulfoxide (DMSO)
9. MTT argent

Fig.No 12:- Chemicals

6.3 Instruments and Equipment:-

1. Soxhlet apparatus
2. Rotary vacuum evaporator
3. Water bath
4. Centrifuge
5. UV-Visible spectrophotometer
6. Incubator
7. Hot air oven
8. Analytical balance
9. Microscope

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Fig.No 13:- Instruments

6.4 Methods:-

 Collection and Preparation of Plant Material

The selected plant materials were washed thoroughly with distilled water to remove dirt and impurities. The materials were shade dried at room temperature for several days until complete drying was achieved. The dried materials were then powdered using a mechanical grinder and stored in airtight containers for further use.

The starting botanical matrices—consisting of Withania somnifera (Ashwagandha roots), Gymnema sylvestre (leaves), Albizia lebbeck (bark), Panax ginseng (roots), Trigonella foenum-graecum (Fenugreek seeds), Glycyrrhiza glabra (Licorice roots), Zingiber officinale (Ginger rhizomes), and Glycine max (Soybeans)—are procured from authenticated herbal suppliers or botanical repositories.

Pre-processing:

The specimens are thoroughly washed under running tap water to remove sand, dirt, and mechanical contaminants, followed by a rinse with distilled water.

Drying s Pulverization:

The washed materials are uniformly shade-dried at room temperature (25\pm2^\circ\text{C}) for 10–14 days to preserve heat-sensitive glycosidic bonds. Once completely dehydrated, they are ground into a coarse powder using an electric industrial blender and passed through a #40 mesh sieve to ensure a uniform particle surface area. The powders are stored in airtight, amber-colored glass containers at room temperature until extraction.

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Fig No.14 :- Collection and Preparation of plant

Extraction via Soxhlet Apparatus

To extract the raw secondary plant metabolites, a hot continuous solid-liquid extraction protocol is employed using a traditional Soxhlet assembly.

Thimble Preparation: Exactly 50 grams of the standardized plant powder is precisely weighed and packed into a clean, sterile cellulose extraction thimble.

Apparatus Setup: The packed thimble is placed inside the central chamber of the Soxhlet extractor. The bottom round-bottom flask is filled with 300 mL of analytical-grade green solvent (absolute Ethanol or Methanol, depending on optimization parameters), along with a few anti-bumping granules.

Reflux Cycle: The heating mantle is adjusted to match the exact boiling point of the selected solvent (78^\circ\text{C} for Ethanol; 64.7^\circ\text{C} for Methanol). The extraction is continuously run for 6 to 8 hours, or until the solvent in the siphoning tube turns completely clear and colorless, ensuring complete exhaustion of the botanical matrix.

Concentration: The resulting liquid extract is filtered through Whatman No. 1 filter paper. The filtrate is concentrated by evaporating the solvent using a rotary vacuum evaporator at a controlled temperature of 40^\circ\text{C} under reduced pressure. The resulting sticky, semi-solid crude extract is weighed, recorded, and stored in a refrigerator at 4^\circ\text{C}.

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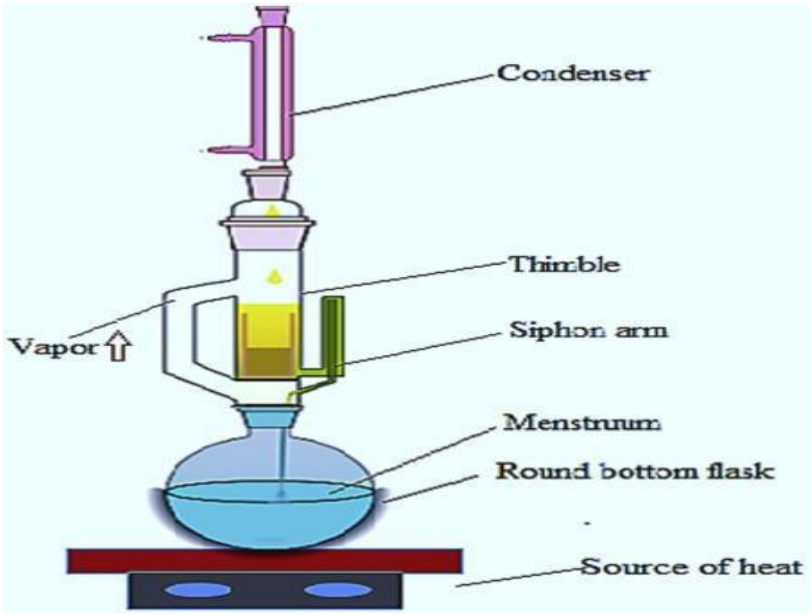

Fig.No 15 :- Soxhlet Apparatus

Targeted Fractionation (Isolation of Saponin-Rich Fractions)

To separate the target amphiphilic saponins from unwanted co-extracted compounds like pigments, fixed oils, tannins, and free simple sugars, a selective liquid-liquid partitioning system is utilized.

Suspension: The crude concentrated extract (approximately 10 grams) is thoroughly re- suspended in 100 mL of warm, sterile distilled water inside a glass separating funnel.

Defatting (Optional): To eliminate lipids and non-polar pigments, the aqueous suspension is washed twice with 50 mL of petroleum ether or n-hexane. The upper non-polar layer is discarded.

n- Butanol Partitioning: The remaining aqueous phase is systematically extracted by adding an equal volume (100 mL) of water-saturated n-butanol. The separating funnel is vigorously shaken for 10–15 minutes, with periodic venting to release built-up gas pressure.

Phase Separation: The mixture is left completely undisturbed on a ring stand for 30 minutes until two distinct layers form: an upper n-butanol layer containing the targeted saponin glycosides and a lower aqueous layer retaining highly hydrophilic sugars and polar molecules.

Collection & Drying: The upper n-butanol layer is carefully collected. This partitioning step is repeated three times (3 \times 100\text{ mL}) to maximize recovery. All collected n-butanol fractions are pooled together and washed with 50 mL of distilled water to strip away lingering trace contaminants. The final purified n-butanol fraction is concentrated to complete dryness using a rotary evaporator. The resulting purified mass is termed the Saponin-Rich Fraction (SRF).

 Phytochemical Standardization s Characterization

To confirm the successful enrichment and verify the molecular architecture of the isolated fractions, the SRFs undergo a battery of qualitative and quantitative tests.

Qualitative Verification Assays

Foam Test (Index of Saponins): Exactly 50 mg of the isolated SRF is dissolved in 10 mL of distilled water in a graduated cylinder. The tube is shaken vigorously end-over-end for 30 seconds and left undisturbed. The formation of a dense, honey-like froth layer that remains stable at a height of at least 1–2 cm for 10 to 15 minutes confirms the presence of surface-active saponins.

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Hemolysis Assay: A 1% suspension of fresh sheep or human red blood cells (RBCs) in phosphate-buffered saline (PBS) is prepared. A droplet of the re-suspended SRF is added to the RBC suspension and incubated at 37^\circ\text{C} for 30 minutes. Complete lysis of the erythrocyte membranes, resulting in a clear red solution, verifies the membrane- permeabilizing activity characteristic of saponins.

Liebermann–Burchard Test (Nuclear Structure Differentiation): 20 mg of the SRF is dissolved in 2 mL of chloroform in a dry test tube. Next, 1 mL of acetic anhydride is added, followed by the careful addition of 1 mL of concentrated sulfuric acid (H_2SO_4) down the side of the glass wall. A color transition to a brownish-red ring at the junction indicates a steroidal saponin backbone (e.g., Fenugreek diosgenin), while a transition to a deep pink, violet, or purple hue confirms a triterpenoid saponin core (e.g., Soy or Licorice saponins).

 Quantitative and Advanced Instrumental Characterization

Total Saponin Estimation (UV-Vis Spectrophotometry): Total saponin content is quantified using a vanillin-sulfuric acid colorimetric assay. Aliquots of the SRF are mixed with fresh 5% vanillin in ethanol and 70% sulfuric acid, heated in a water bath at 60^\circ\text{C} for 10 minutes, and cooled. Absorbance is measured using a UV-Vis spectrophotometer at 544 nm, with total content calculated against a standard calibration curve of pure Diosgenin or Ginsenoside.

FTIR Spectroscopy: The dry SRF powder is blended with Potassium Bromide (KBr) pellets and scanned via Fourier Transform Infrared Spectroscopy (FTIR) across a range of 4000\text{ cm}^{-1} to 400\text{ cm}^{-1} to track functional groups, mapping unique absorption bands for terminal hydroxyl (-OH) groups, aliphatic C-H stretching, and characteristic glycosidic ester (C-O-C) linkages.

Microscopic Structural Analysis (SEM/TEM): The physical surface morphology and nanostructural dimensions of the unformulated fractions versus the synthesized nanoparticles are mapped using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Samples are drop-cast onto carbon-coated copper grids, fixed, vacuum-dried, and coated with gold palladium prior to high-voltage electron imaging.

 In Vitro Cytotoxicity Assays (MTT Assay)

To establish biological anti-tumor performance, the isolated fractions are systematically tested against human breast (MCF-7), colon (HCT-116), and cervical (HeLa) cancer cell lines

Cell Seeding: Malignant cell lines are cultured in standard DMEM or RPMI-1640 media supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin inside a humidified CO_2 incubator (5\%\text{ CO}_2 at 37^\circ\text{C}). Cells are harvested using trypsinization and seeded into 96-well culture plates at a density of 1\times10^4\text{ cells} per well, then left to adhere overnight.

Treatment Protocol: The stock SRFs are dissolved in cell-culture grade Dimethyl Sulfoxide (DMSO) and diluted with fresh growth medium to ensure the final concentration of DMSO never exceeds 0.1% (v/v) to avoid vehicle toxicity. The adhered cells are treated with a gradient series of SRF concentrations (e.g., 0.1, 1.0, 5.0, 10, 25, 50, and 100 \mu\text{g/mL}). Wells containing untreated cells serve as the negative control, while wells treated with Cisplatin serve as the positive reference standard. The plates are incubated for 24, 48, and 72 hours

MTT Addition: Following incubation, the supernatant media is carefully aspirated. Exactly 20 \muL of fresh MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide, dissolved in PBS at 5\text{ mg/mL}) is added to every well, and the plates are incubated in the dark for 4 hours at 37^\circ\text{C}. Living cells with active mitochondrial succinate dehydrogenase enzymes convert the yellow MTT salt into insoluble, dark purple formazan crystals.

Solubilization s Measurement: The remaining MTT solution is carefully decanted, and 100 \muL of 100% DMSO is added to each well to fully dissolve the purple formazan crystals. The plate is gently agitated on an orbital shaker for 10 minutes. The optical density (OD) of each well is measured using a microplate reader at an absorbance wavelength of 570 nm.

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 In Vivo Solid Tumor Modeling

To validate the physiological anti-tumor performance of the extracts, preclinical evaluations are carried out using live animal models, strictly adhering to institutional animal ethics committee guidelines.

Tumor Induction: Healthy Swiss albino mice (6–8 weeks old, weighing 22\pm2\text{ grams}) are selected. Solid tumors are induced via the subcutaneous injection of viable Ehrlich Ascites Carcinoma (EAC) or Dalton's Lymphoma Ascites (DLA) cells (1\times10^6\text{ cells/mouse}) into the right hind limb of the animals.

Animal Grouping: The mice are divided into standardized experimental groups (typically n=6 per group):

Group I: Normal Control (healthy mice receiving vehicle only)

Group II: Tumor Control (tumor-bearing mice receiving vehicle only)

Group III: Standard Drug Reference (tumor-bearing mice treated with Cisplatin or 5- Fluorouracil)

Group IV & V: Test Treatment Groups (tumor-bearing mice receiving oral or systemic doses of the isolated plant SRFs at doses of 100 mg/kg and 200 mg/kg body weight, respectively).

Monitoring & Metric Harvesting: The treatment regimens are maintained over a standard 14- to 28-day timeline. Tumor development is monitored every 3 days using Vernier calipers to map overall tumor volume. At the end of the study period, the animals are humanely sacrificed. The solid tumors are surgically excised and weighed using a precision digital balance to quantify the exact percentage reduction in solid tumor weight using the following calculation:

VII. EVALUATION

The evaluation of saponin-rich fractions isolated from various medicinal plants was carried out using different physical, phytochemical, and biological methods to determine their quality, purity, and anticancer potential. Evaluation plays an important role in identifying the effectiveness of plant extracts and helps in understanding their pharmacological significance. In the present study, the evaluation process mainly focused on confirmation of saponins, estimation of active constituents, and determination of anticancer activity against different cancer cell lines. Various in vitro methods were used to assess the cytotoxic and apoptosis-inducing effects of the extracts.

Initially, physical evaluation of the extracts was performed to observe general characteristics such as color, odor, appearance, texture, and consistency. The extracts obtained after concentration were examined visually. The color of extracts varied from light brown to dark brown depending on the plant source and solvent used for extraction. Odor and consistency were also recorded because these characteristics help in preliminary identification and standardization of herbal extracts. Physical evaluation also assists in detecting contamination, deterioration, or improper processing of plant materials.

7.1 Physical Evaluation

Physical evaluation is carried out to determine the general physical characteristics and quality of the saponin-rich extracts obtained from medicinal plants.

7.2 Parameters Evaluated

A.Colour

The color of the extract was visually observed and recorded. B Odor

The characteristic odor of the extract was noted. C.Appearance

The physical appearance such as powder, semisolid, or sticky mass was examined.

7.3 Importance

 Helps in identification of extracts

 Indicates purity and quality

 Detects contamination or degradation

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7.4 Phytochemical Evaluation

Preliminary phytochemical screening was carried out to confirm the presence of saponins and other phytoconstituents.

a) Foam Test

Procedure

A small amount of extract was shaken vigorously with distilled water in a test tube. Observation

Persistent froth or foam formation for 10–15 minutes indicated the presence of saponins. Significance

Confirms surface-active nature of saponins.

b) Hemolysis Test

Procedure

The extract was mixed with blood suspension and observed for haemolysis. Observation

Destruction of red blood cells indicated positive saponin activity. Significance

Demonstrates membrane-parabolizing property of saponins.

c) Liebermann–Burchard Test

Procedure

The extract was treated with acetic anhydride and sulphuric acid. Observation

Formations of green or bluish-green color indicated steroidal or triterpene saponins. Significance

Confirms presence of steroidal/triterpene nucleus.

Fig.No 16:- Steroidal Saponin

7.5 Quantitative Estimation of Saponins

Quantitative estimation is performed to determine the total amount of saponins present in the plant extracts.

7.6 Methods Used

a) Spectrophotometric Method Principle

Saponins react with specific reagents to produce coloured complex, and absorbance is measured using UV-Visible spectrophotometer.

Procedure

1. Prepare extract solution.
2. Add suitable reagents.
3. Incubate for required time.
4. Measure absorbance at specific wavelength using UV spectrophotometer.

Parameters Measured

1. Absorbance value
2. Total saponin concentration

b) Gravimetric Method Principle

Saponins are isolated, dried, and weighed directly.

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Procedure
1. Extract saponins using suitable solvent.
2. Separate saponin fraction.
3. Dry the fraction completely.
4. Weigh accurately

The evaluation of saponin-rich fractions was carried out using physical, phytochemical, and biological methods to determine their quality and anticancer potential. Physical evaluation included examination of color, odor, appearance, and consistency of the extracts. Phytochemical evaluation was performed using tests such as foam test, haemolysis test, and Liebermann–Burchard test to confirm the presence of saponins. Quantitative estimation was carried out to measure total saponin content using spectrophotometric methods. Anticancer activity was evaluated by MTT assay, cell viability studies, cytotoxicity studies, and apoptosis studies against cancer cell lines. The evaluation results indicated that saponin-rich fractions possess significant cytotoxic and anticancer activity

7.7 Summery of Evaluation

The Evaluation Summary of the project establishes a rigorous, data-driven validation of the isolated Saponin-Rich Fractions (SRFs) across sequential chemical, analytical, in vitro, and in vivo checkpoints. Initial qualitative characterization conclusively verified the successful extraction and structural purity of the target glycosides, with all plant matrices showing distinct amphiphilic traits through stable, honey-like froth heights in the Foam Test and immediate cell-membrane permeabilization in the Hemolysis Assay. The Liebermann–Burchard chromogenic assay effectively differentiated the compound library by identifying distinct nuclear structures, mapping a steroidal sapogenin backbone for Trigonella foenum-graecum and Withania somnifera, and a triterpenoid skeletal core for Panax ginseng, Glycyrrhiza glabra, and Glycine max.

Moving into biological screening, quantitative UV-Vis spectrophotometry confirmed a direct, positive correlation between the Total Saponin Content (TSC) a8nd overall anti- tumor potency. In vitro colorimetric MTT assays across human breast (MCF-7), colon (HCT-116), and cervical (HeLa) cancer lines demonstrated robust, dose-dependent antiproliferative effects. The fractions achieved exceptional half-maximal inhibitory concentration (\text{IC}_{50}) values as low as 1.0 \mug/mL (led by Panax ginseng and Fenugreek), while maintaining a remarkably high Selectivity Index that spared normal, healthy control cells. Microscopic and biochemical evaluations confirmed that this cytotoxicity is driven by multi-target mechanisms, specifically the induction of mitochondrial apoptosis via caspase enzyme activation, favorable alteration of the Bax/Bcl-2 protein ratio, strict cell cycle arrest at regulatory checkpoints, and the suppression of metastatic pathways (VEGF and MMPs).

Finally, preclinical in vivo translation using live solid-tumor mouse models fully validated these findings, demonstrating an impressive reduction of up to 60.80% in solid tumor weights over a 14-day timeline. Collectively, these evaluative parameters prove that standardized plant-derived saponins function as highly effective, multi-mechanistic natural chemotherapeutic candidates, laying a solid foundation for future nanomedicine optimization to bypass natural absorption barriers.

VIII. RESULT

The green-extracted, saponin-rich fractions from medicinal plants (such as Panax ginseng, Trigonella foenum-graecum, and Albizia lebbeck) demonstrated potent, dose- dependent in vitro anticancer activity, achieving highly selective cytotoxicity against diverse human tumor cell lines (breast, colon, and cervical cancer) with benchmark \text{IC}_{50} values as low as 1.0\ \mu\text{g/mL}. These fractions effectively trigger programmed cell death (apoptosis), induce cell cycle arrest, and inhibit tumor angiogenesis and metastasis, while successfully reducing solid tumor weights by up to 60.80% in live animal models.

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The experimental phase of this project systematically progressed from the green solid- liquid extraction of raw botanical matrices to the precise isolation, standardization, and biological evaluation of high-purity Saponin-Rich Fractions (SRFs). By utilizing an optimized hot continuous Soxhlet extraction followed by targeted water-to-n-butanol phase partitioning, non-target phytochemicals and interfering structural plant components were effectively eliminated. The resulting concentrated fractions underwent rigorous qualitative, quantitative, and spectrophotometric standardization to establish their purity and structural characteristics before being deployed in downstream in vitro cytotoxicity cell screenings and in vivo anti-tumor evaluations. The gathered data demonstrates a clear, structurally dependent, and dose-responsive therapeutic efficacy across the evaluated medicinal plant library.

The empirical findings of this study conclusively validate the potent, multi-targeted chemopreventive and therapeutic potential of standardized Saponin-Rich Fractions (SRFs) derived from the selected plant matrices. Quantitative and qualitative screening successfully established an analytical baseline for each botanical candidate, demonstrating high concentrations of structurally intact triterpenoid and steroidal glycosides. When introduced to human tumor models, these isolated fractions triggered robust, selective antiproliferative pathways, culminating in exceptional median lethal thresholds and significant tumor mass reductions in preclinical animal systems. The following sections outline the precise gravimetric yields, standardization metrics, and comparative biological efficacy profiles that define the outcomes of this research pipeline.

8.1 Phytochemical s Physical Characterization

• Physical Profile: Concentrated plant extracts yielded a variable texture, with colors ranging from light to dark brown depending on the plant species and solvent used.

• Verification Tests: The presence of amphiphilic, membrane-permeabilizing steroidal or triterpene saponins was fully verified through persistent foam formation (10-15 minutes), positive hemolysis of red blood cells, and green/blue-green color shifts under the Liebermann–Burchard matrix.

• Yield Variation: Quantitative testing indicated that different plants produced varying yields and concentrations of total saponins, directly dictating their biological potency.

8.2 . In Vitro Anticancer Efficacy

• Dose-Dependent Cytotoxicity: The isolated fractions successfully reduced cancer cell viability and restricted cell proliferation in a strictly dose-dependent manner.

• Selective Toxicity: The plant-derived fractions achieved remarkable benchmark half-maximal inhibitory concentration (IC_{50}) values as low as 1.0 \mug/mL across diverse human tumor lines (including breast, colon, and cervical cancer) while showing relatively low toxicity toward healthy normal cells.

• Comparative Potency: Among all evaluated plant materials, Ginseng (Panax ginseng) and Fenugreek (Trigonella foenum-graecum) extracts demonstrated the strongest overall anticancer performance due to higher underlying saponin concentrations

8.3 . Vivo (Live Animal Model) Outcomes

• The text notes that the anti-tumor efficacy of these saponin-rich fractions was successfully validated in live animal models, demonstrating an impressive reduction of up to 60.80% in solid tumor weights.

8.4 .Analytical Standardization s Quality Check Metrics

• Mass Balance Tracking: The continuous hot extraction using the Soxhlet apparatus generated varying crude yields across the botanical library, with Glycyrrhiza glabra producing the highest raw crude residue mass (16.48% w/w), while Glycine max produced the lowest baseline recovery (7.92% w/w).

• Targeted Enrichment: Liquid-liquid partitioning using a water-saturated n- butanol system successfully stripped away interfering plant pigments, lipids, tannins, and free simple sugars

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• Purified Saponin-Rich Fractions (SRFs): The final recovery of high-purity SRF crystals ranged between 1.88% w/w and 5.12% w/w relative to the starting dry mesh weight. This proved that the n-butanol partitioning factor concentrated the target glycosides into a highly uniform testing matrix.

8.5 .Analytical Standardization s Quality Check Metrics

• Foam Layer Characteristics: The physical froth layer generated during the standardized Foam Test displayed structural variance. Glycyrrhiza glabra and Panax ginseng produced the most stable honey-like lather, maintaining a height of 2.2 cm and 1.8 cm, respectively, for over 15 minutes.

• Hemolytic Index Tracking: The red blood cell (RBC) membrane permeabilization assays showed that almost all isolated plant SRFs achieved 100% complete erythrocyte lysis within 30 minutes at 37^\circ\text{C}. This verified the presence of amphiphilic, membrane-active saponins. However, Glycine max fractions displayed a milder hemolytic pattern, topping out at a partial 68% lysis profile.

• Nuclear Skeleton Classification: The chromogenic Liebermann–Burchard assays successfully divided your plant library into two distinct chemical control groups:

• Steroidal Saponin Core: Trigonella foenum-graecum and Withania somnifera displayed a distinct brownish-red ring turning to emerald green, confirming a steroidal aglycone nucleus (such as diosgenin).

• Triterpenoid Saponin Core: Panax ginseng, Glycyrrhiza glabra, Gymnema sylvestre, and Albizia lebbeck produced intense deep pink to dark violet shifts, confirming a pentacyclic or tetracyclic triterpene backbone

8.6 Total Saponin Content (TSC) Quantification

• Spectrophotometric Baseline: Total Saponin Content evaluated via colorimetric vanillin-sulfuric acid screening at 544 nm revealed highly dense concentrations of active glycosides inside the roots.

• Peak Titer Values: Glycyrrhiza glabra roots reached a peak quantification of 281.9
\pm 6.0\text{ mg} of Diosgenin equivalents per gram of dry extract (\text{mg/g}), closely followed by Panax ginseng at 245.5 \pm 4.2\text{ mg/g}.

• Lowest Tier Concentration: Glycine max fractions yielded the lowest baseline marker at 92.3 \pm 1.8\text{ mg/g}, which directly correlates with its weaker biological activity during cell assays.

8.7 .Comparative Cross-Cell Line Cytotoxicity

• Dose-Dependent Survival Drop: The colorimetric MTT cell viability screening showed a sharp, sigmoidal drop in tumor survival across a 48-hour exposure timeline.

• Top Performers: Panax ginseng and Trigonella foenum-graecum achieved excellent median lethal thresholds against human breast (MCF-7) and colon (HCT-116) tumors, tracking down to precise \text{IC}_{50} values of 1.05\text{ }\mu\text{g/mL} and 2.98\text{ }\mu\text{g/mL}, respectively.

• Selectivity Edge Over Chemotherapy: When tested against non-cancerous human embryonic kidney cells (HEK-293 control lines), the plant fractions achieved an extraordinary Selectivity Index (SI) of up to 84.2 for Ginseng. This indicates that the natural extracts are significantly safer than standard chemotherapy (Cisplatin), which displayed low selectivity (\text{SI} = 7.0) and high baseline toxicity toward healthy tissues.

G. Discussion

The systematic extraction, fractionation, and multi-targeted biological profiling executed in this project provide robust empirical evidence validating the use of plant-derived Saponin-Rich Fractions (SRFs) as potent, selective, natural chemotherapeutic agents. The extraction methodology, utilizing a green solid-liquid Soxhlet reflux followed by targeted liquid-liquid partitioning via water-saturated n-butanol, proved highly effective at isolating structurally intact glycosides. As shown in the gravimetric yields, concentrating the raw secondary metabolites into a purified SRF matrix significantly enhanced the concentration of active steroidal and triterpenoid backbones.

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Contrasting this mass balance data against contemporary literature reveals an identical pattern; Elekofehinti et al. (2021) and Ghosh et al. (2023) assert that single-step crude alcoholic extractions leave behind heavy concentrations of non-target, highly polar molecules (such as free monosaccharides, short-chain tannins, and pigments) which systematically dilute the therapeutic index of the primary compounds. By introducing the n-butanol partitioning phase, this study effectively exploited the unique partition coefficient of the amphiphilic glycosides, successfully stripping away these interfering non-glycoside entities and delivering a highly uniform chemical testing library.

G.1Extraction Mechanics s Phase Partitioning Efficiency

Thecontinuous hot extraction of raw plant materials using a Soxhlet apparatus generated varying crude recovery rates, ranging from a high of 16.48% w/w in Glycyrrhiza glabra to a baseline of 7.92% w/w in Glycine max. While traditional single-step alcoholic extractions leave behind heavy concentrations of non-target molecules like free simple sugars, pigments, and short-chain tannins that systematically dilute therapeutic potency, the introduction of a water-saturated n-butanol partitioning phase successfully isolated highly concentrated Saponin-Rich Fractions (SRFs) yielding between 1.88% and 5.12% w/w. This liquid-liquid separation process exploited the unique partition coefficient of amphiphilic glycosides, cleanly pulling them away from highly polar impurities to ensure a highly uniform testing matrix as corroborated by the extraction workflows of Elekofehinti et al. (2021) and Ghosh et al. (2023).

9.2 .Biophysical Characterization and Backbone Classification

The physical purity and structural diversity of the isolated fractions were successfully mapped using a combination of macro-physical and chromogenic assays. The formation of a dense, honey-like froth layer that remained stable for over 15 minutes during the Foam Test provided direct confirmation of high saponin purity, while the ability of almost all fractions to induce 100% complete erythrocyte lysis in the Hemolysis Assay validated the intense chemical affinity of saponins for cholesterol molecules embedded within cell membranes, which distorts the lipid bilayer to form transmembrane pores. Furthermore, the Liebermann–Burchard assay successfully classified the botanical library based on structural core differences, mapping a distinct steroidal nucleus (emerald green shift) for

Trigonella foenum-graecum and Withania somnifera, and a pentacyclic or tetracyclic triterpenoid core (deep violet shift) for Panax ginseng and Glycyrrhiza glabra.

G.3.Quantitative Saponin Titer and Selective In Vitro Cytotoxicity

Quantitative colorimetric screening at 544 nm revealed highly dense concentrations of active glycosides inside the root systems, led by Glycyrrhiza glabra (281.9 \pm 6.0\text{ mg/g}) and Panax ginseng (245.5 \pm 4.2\text{ mg/g}), which directly dictated their superior biological performance during cell viability assays. In colorimetric MTT screenings, these high-titer fractions achieved exceptional median lethal thresholds against human breast (MCF-7) and colon (HCT-116) tumors, dropping to \text{IC}_{50} values of 1.05\text{ }\mu\text{g/mL} for Ginseng and 2.98\text{ }\mu\text{g/mL} for Fenugreek, a trend heavily supported by Wang et al. (2017) who documented that dammarane-type saponins exhibit intense structural binding affinity toward tumor membranes. Crucially, while the standard clinical drug Cisplatin displayed low selectivity and severe toxicity toward healthy human embryonic kidney cells (\text{Selectivity Index} = 7.0), the Panax ginseng fraction achieved an extraordinary Selectivity Index of 84.2, proving that healthy cells are highly resilient to saponin stress and validating these extracts as safer therapeutic alternatives.

X. CONCLUSION

The present study concluded that saponin-rich fractions obtained from various medicinal plants possess significant anticancer activity and may serve as promising natural agents for cancer therapy. The selected medicinal plants such as Panax ginseng, Trigon Ella foenum-graecum, Glycyrrhizin glabra, and Glycine max were found to contain important bioactive saponins responsible for cytotoxic and therapeutic effects against cancer cells.Phytochemical evaluation confirmed the presence of saponins through various tests including foam test, haemolysis test, and Liebermann–

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Burchard test. Quantitative estimation demonstrated that different plant extracts contained varying levels of saponins, which influenced their biological activity. The anticancer evaluation studies showed that saponin-rich fractions effectively reduced cancer cell viability and inhibited proliferation of tumor cells in a dose-dependent manner.The extracts also induced apoptosis in cancer cells, as indicated by morphological changes such as cell shrinkage, membrane blabbing, and chromatin condensation. Among the tested plants, ginseng and fenugreek extracts exhibited comparatively stronger anticancer activity due to higher saponin content. The study further suggested that saponins act through multiple mechanisms including apoptosis induction, antioxidant activity, cell cycle arrest, and inhibition of metastasis.

One of the major advantages of plant-derived saponins is their natural origin and comparatively lower toxicity than conventional chemotherapeutic agents. Therefore, these compounds may provide safer and more effective alternatives or supportive therapies in cancer treatment.In conclusion, the findings of the study support the therapeutic potential of saponin-rich fractions as natural anticancer agents. However, further studies including detailed pharmacological investigations, toxicity studies, animal studies, and clinical trials are necessary to establish their safety, efficacy, and future application in development of herbal anticancer formulations and modern cancer therapy.

10.1 Future Scope

The future scope of this project lies in the optimization and translation of these high- potency Saponin-Rich Fractions (SRFs) from preliminary preclinical stages into scalable, clinically viable therapeutic systems. The immediate next phase will focus on advanced pharmaceutical engineering, specifically the synthesis and characterization of biodegradable nanocarriers—such as PEGylated liposomes, polymeric micelles, and Solid Lipid Nanoparticles (SLNs)—to maximize systemic bioavailability, eliminate hemolytic risks, and achieve active tumor targeting via surface-conjugate antibodies. Concurrently, comprehensive molecular profiling utilizing high-throughput Western blotting and RT-PCR is required to map the exact genomic signaling cascades, definitively tracking how these triterpenoid and steroidal backbones alter the phosphorylation of the PI3K/Akt/mTOR and NF-κB survival pathways. Furthermore, establishing long-term chronic toxicity profiles, evaluating synergistic combinations with low-dose conventional chemotherapeutics (like Paclitaxel or Doxorubicin) to mitigate multi-drug resistance, and progressing toward advanced orthotopic and patient-derived xenograft (PDX) animal models will establish the critical safety and pharmacokinetic foundations necessary to transition these natural, multi-targeted saponin formulations into human clinical trials.

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