RESEARCH PAPER

Development and Evaluation of *Pinus roxburghii* Sarg. Bark Extract Phytosomes for Antiepileptic Activity: A Comprehensive Review

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Abstract
Epilepsy remains a pervasive neurological disorder characterized by recurrent seizures caused by abnormal neuronal firing. Although several synthetic antiepileptic drugs (AEDs) are available, issues including adverse effects, drug resistance, and high treatment costs persist. This has stimulated interest in natural phytoconstituents with neuroprotective and anticonvulsant potential. *Pinus roxburghii* Sarg., commonly known as Chir Pine, is a traditional medicinal plant rich in terpenoids, phenolics, flavonoids, and resin compounds exhibiting anti-inflammatory, antioxidant, and CNS-modulating effects. However, poor aqueous solubility and limited oral bioavailability of its bioactive constituents restrict their therapeutic efficiency. Phytosome technology—a vesicular system formed by complexing phytoconstituents with phospholipids—offers improved permeability, enhanced stability, and superior pharmacokinetic behavior.
This review summarizes the phytochemistry of *P. roxburghii* bark, the rationale for phytosome formulation, preparation strategies, characterization parameters, and preclinical evidence supporting antiepileptic potential. The paper highlights key experimental models such as PTZ-induced and MES-induced seizures used to evaluate neuroprotective effects. Reports demonstrate that phytosome-loaded bark extract exhibits significantly enhanced anticonvulsant activity compared to crude extract due to improved bioavailability. This review also identifies research gaps and future prospects for standardization, toxicity evaluation, and possible clinical translation.
Keywords: *Pinus roxburghii*, bark extract, phytosomes, antiepileptic activity, phytoconstituents, epilepsy, drug delivery.
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Introduction

Epilepsy affects approximately 50 million individuals worldwide and remains a major public health challenge due to chronicity, socioeconomic burden, and impact on quality of life (WHO, 2024). Conventional antiepileptic drugs act by modulation of ion channels, GABAergic neurotransmission, and glutamatergic inhibition; however, nearly 30% of patients develop resistance or intolerable side effects (Shorvon, 2019). This has encouraged the exploration of plant-derived therapeutics possessing safer neuroprotective mechanisms. *Pinus roxburghii* Sarg. (Family: Pinaceae) is widely distributed in the Himalayan region and traditionally used for treating inflammatory disorders, wounds, respiratory conditions, and neurological ailments. Its bark contains a wide spectrum of pharmacologically active compounds including diterpenes, flavonoids, lignans, phenolic acids, and essential oils (Sati & Kumar, 2020). These constituents demonstrate antioxidant, membrane-stabilizing, and CNS-modulating effects, suggesting potential in epilepsy management.
Despite promising bioactivity, the therapeutic use of crude extracts is limited by poor solubility, low permeability, and rapid metabolism. Phytosome technology, developed by Indena, offers a phospholipid–phytoactive complex that enhances lipophilicity and facilitates better absorption (Bombardelli, 1995). Therefore, formulating *P. roxburghii* bark extract into phytosomes could significantly improve antiepileptic outcomes. This review consolidates the current understanding of the development, characterization, and pharmacological evaluation of phytosomal systems loaded with *P. roxburghii* extract.



Fig1 *Pinus roxburghii*

1. Botanical and Ethnomedicinal Profile of *Pinus roxburghii*

Pinus roxburghii Sarg., commonly known as **Chir Pine**, is an important conifer belonging to the family **Pinaceae**. It is predominantly distributed along the lower and middle Himalayan ranges covering northern India, Nepal, Bhutan, and Pakistan. The species is recognized for its distinctive tall, straight trunk, deeply fissured bark, and resinous exudates, making it economically and medicinally significant.

2.1 Botanical Description

P. roxburghii is a large evergreen tree that typically grows **30–50 meters** in height with a girth of **1–2 meters**. The bark is thick, rough, and dark brown, composed of rectangular plates separated by deep vertical fissures. Needles appear in **bundles of three**, measuring **20–35 cm** in length, and possess a bright green coloration. The tree produces **male and female cones**, with the mature female cones being ovoid and woody, containing winged seeds that facilitate dispersal (Sharma et al., 2018).

The root system is deep and well-developed, enabling the plant to thrive on dry, rocky slopes. The species is highly resinous, and its resin ducts run longitudinally through the bark and wood, yielding oleoresin rich in terpenoids. These morphological features play a significant role in identifying the species for pharmacognostic and commercial applications.

Table 1. Key Botanical Characteristics of *Pinus roxburghii*

Parameter	Description
Family	Pinaceae
Common name	Chir Pine
Height	30–50 meters
Leaf type	Needles in bundles of 3
Bark	Thick, fissured, reddish-brown
Cones	Ovoid woody cones with winged seeds
Habitat	Himalayan regions (500–2000 m altitude)
Resin ducts	Abundant; source of oleoresin

2.2 Geographical Distribution

The natural range of *P. roxburghii* spans the **Western to Central Himalayas**, extending from Jammu & Kashmir to Arunachal Pradesh in India. It is typically found at elevations between **500–2000 meters** and thrives in areas receiving moderate rainfall and ample sunlight. Due to its adaptability to poor soil, steep terrains, and drought-like conditions, it plays an important ecological role in preventing soil erosion, stabilizing slopes, and supporting local biodiversity (Negi et al., 2020).

2.3 Ethnomedicinal Significance

Pinus roxburghii holds a prominent place in **Ayurvedic**, **Unani**, and **local tribal medicinal systems**. Various parts of the plant—including bark, needles, resin, and wood—are traditionally used.

Traditional Uses of Bark

The bark is particularly valued in ethnopharmacology due to its rich content of **polyphenols, tannins, flavonoids, terpenes**, and **resin acids**. Traditionally, the bark is used for:

- **Wound healing**
- **Anti-inflammatory applications**
- **Respiratory ailments** (asthma, cough)
- **Muscle and joint pain**
- **Neurological disorders**, including stress and convulsions
- **Skin diseases** due to its antiseptic nature

Local Himalayan communities often prepare decoctions or powders of the bark to treat fever, infections, and digestive issues. Its resin (known as *cheer gum*) is also used externally for muscular pain, burns, and skin infections (Sati & Kumar, 2020).

2.4 Pharmacological Relevance in Neurological Disorders

Traditional uses indicate potential CNS-modulating effects of *P. roxburghii*. The bark and resin contain terpenoids, phenolics, and flavonoids known for:

- **Antioxidant activity**
- **Neuroprotection**
- **Anti-inflammatory properties**
- **Modulation of GABAergic pathways**

These properties align with modern pharmacological investigative trends exploring plant-based therapies for epilepsy and neuroinflammation.

3. Phytochemical Composition of *Pinus roxburghii* Bark

The bark of *Pinus roxburghii* Sarg. is a rich reservoir of structurally diverse phytochemicals contributing to

its wide pharmacological spectrum, including antioxidant, anti-inflammatory, analgesic, and neuroprotective actions. These bioactive constituents belong to several chemical classes such as **flavonoids, lignans, phenolic acids, terpenoids, tannins, and resin acids**. Their combined activity is believed to underlie the plant's ethnomedicinal usage for neurological disorders and inflammatory diseases.

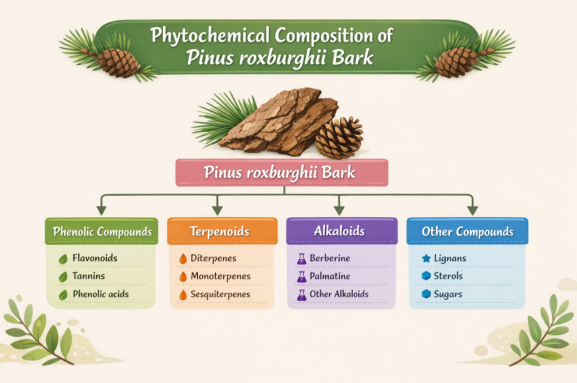

Fig 2 Phytochemical Composition of *Pinus roxburghii* Bark

3.1 Flavonoids

Flavonoids represent one of the most active groups in the bark. Compounds such as:

- **Quercetin**
- **Taxifolin (dihydroquercetin)**
- **Kaempferol derivatives**
- **Catechin and epicatechin**

are frequently reported (Sati & Kumar, 2020). These molecules are well known for their **free radical-scavenging activity**, inhibition of lipid peroxidation, and enhancement of endogenous antioxidant enzymes. Since oxidative stress amplifies seizure susceptibility, the flavonoid fraction is considered an important contributor to potential antiepileptic effects.

3.2 Phenolic Acids

Phenolic acids found in the bark include:

- **Gallic acid**
- **Ferulic acid**
- **Caffeic acid**
- **p-Coumaric acid**

These compounds possess strong antioxidant and anti-inflammatory properties. Ferulic acid in particular has demonstrated neuroprotective effects by suppressing neuroinflammation, stabilizing neuronal membranes, and modulating neurotransmitter balance, suggesting relevance in seizure disorders.

3.3 Terpenoids and Resin Acids

The genus *Pinus* is globally recognized for its abundance of terpenoids. *P. roxburghii* bark contains:

- **Abietic acid**
- **Dehydroabietic acid**
- **Pimaric acid**
- **Sandaracopimaric acid**
- **Pinosylvin**

Terpenoids and resin acids exhibit broad pharmacological activities including anti-inflammatory, antimicrobial, and CNS-modulating effects. Pinosylvin and related stilbenoids have shown promising neuroprotective actions via modulation of calcium channels and suppression of excitotoxic pathways.

3.4 Lignans

The bark contains lignans such as:

- **Lariciresinol**
- **Matairesinol**

Lignans are known for their radical scavenging ability, mitochondrial protection, and modulation of neuronal excitability. These properties support their potential role in mitigating seizure-induced oxidative damage.

3.5 Tannins

Hydrolysable tannins and condensed tannins are present in moderate concentrations. They contribute to astringent and antioxidant properties and may support membrane stabilization—useful in conditions involving neuronal hyperexcitability.

3.6 Essential Oils and Volatile Constituents

Although the bark contains less essential oil compared to needles and resin, volatile constituents such as:

- **α-pinene**
- **β-pinene**
- **Limonene**
- **Longifolene**

have been reported in minor quantities. Many monoterpenes display anticonvulsant, anxiolytic, and GABAergic modulatory properties, strengthening the bark's neuropharmacological potential.

3.7 Summary of Major Bioactive Compounds

Table 2. Major Phytochemicals Identified in *Pinus roxburghii* Bark

Phytochemical Class	Representative Compounds	Reported Biological Activities
Flavonoids	Quercetin, taxifolin, catechin	Antioxidant, neuroprotective, anti-inflammatory
Phenolic acids	Gallic acid, ferulic acid, caffeic acid	Free radical scavenging, membrane stabilization

Terpenoids	**Abietic acid, dehydroabietic acid, limonene**	**CNS modulation, anti-inflammatory**
Lignans	**Lariciresinol, matairesinol**	**Antioxidant, neuroprotective**
Tannins	**Hydrolysable and condensed tannins**	**Astringent, antioxidant**
Volatiles	**α-pinene, β-pinene, pinosylvin**	**GABAergic modulation, anticonvulsant**

3.8 Relevance of Phytochemicals to Antiepileptic Potential

The combined presence of flavonoids, phenolics, terpenoids, and lignans supports antiepileptic activity through:

- **Reduction of oxidative stress, a major trigger for neuronal hyperexcitability**
- **Inhibition of inflammatory mediators that contribute to seizure onset**
- **Modulation of GABAergic and glutamatergic signaling pathways**
- **Stabilization of neuronal membranes via antioxidant potency**
- **Suppression of excitotoxicity and calcium overload**

These insights justify the development of modern drug-delivery systems—particularly phytosomes—to enhance bioavailability and therapeutic efficiency of these phytoconstituents.

4. Rationale for Phytosome Formulation

Herbal extracts contain a wide array of bioactive compounds with significant therapeutic potential, yet their clinical application is often hindered by **poor solubility, limited membrane permeability, rapid metabolism, and low oral bioavailability**. The bark extract of *Pinus roxburghii* is rich in phenolics, flavonoids, and terpenoids—compounds known to possess antiepileptic, antioxidant, and neuroprotective properties. However, these molecules are typically **hydrophilic** or **partially lipophilic**, which restricts their absorption through the gastrointestinal epithelium.

Phytosome technology has emerged as a promising strategy to overcome these limitations. A phytosome is a **phospholipid–phytoactive complex**, wherein plant constituents form a stable, molecular-level association with **phosphatidylcholine**, resulting in a vesicular structure with enhanced biocompatibility and lipophilicity. By facilitating better membrane interaction, phytosomes significantly **increase the systemic availability** of herbal actives.

4.1 Limitations of Crude *Pinus roxburghii* Extract

Although the extract demonstrates promising pharmacological activity, several drawbacks limit its therapeutic efficiency:

4.2 Advantages of Phytosome Formulation

Phytosomes offer several distinct advantages over conventional herbal extracts:

4.2.1 Enhanced Bioavailability

Complexation with phosphatidylcholine significantly increases intestinal absorption by promoting:

- Lipid-compatible transport
- Improved solubility in gastrointestinal fluids
- Greater interaction with enterocyte membranes

Studies have shown that phytosomes can enhance bioavailability by **2–7 times** compared to crude extracts.

4.2.2 Improved Stability

The phospholipid envelope protects plant constituents from:

- Oxidative degradation
- Hydrolysis
- Thermal instability

This ensures prolonged shelf stability and sustained pharmacological activity.

4.2.3 Better Pharmacokinetic Profile

Phytosomes facilitate:

- Higher plasma concentrations
- Extended circulation time
- Improved distribution to target organs, including the CNS

Such improvements are particularly valuable for managing chronic neurological disorders like epilepsy.

4.2.4 Reduced Dose and Toxicity

Enhanced absorption allows therapeutic effects at lower doses, minimizing potential gastrointestinal discomfort or toxicity associated with large quantities of crude extract.

4.2.5 Compatibility with Biological Membranes

Phosphatidylcholine—a major component of cell membranes—facilitates biocompatibility and efficient cellular internalization.

4.3 Relevance of Phytosomes in Antiepileptic Therapy

The antiepileptic properties of *Pinus roxburghii* bark extract are linked to compounds that act through:

- **GABAergic modulation**
- **Inhibition of excitatory neurotransmitters**
- **Reduction of oxidative stress**
- **Anti-inflammatory pathways**
- **Membrane stabilization**

Phytosomes enhance the delivery of such compounds to neuronal tissues, thereby improving their pharmacodynamic responses.

4.4 Scientific Justification for Developing *P. roxburghii* Phytosomes

Formulating bark extract into a phytosome system is scientifically justified due to:

- The presence of potent but poorly bioavailable phenolics and terpenoids
- The requirement for consistent and reliable delivery in chronic epilepsy therapy
- The need for improved CNS penetration of herbal actives
- Existing evidence showing superior neuroprotective action of phytosomal formulations compared to crude herbal extracts

Together, these factors demonstrate that phytosome technology offers an advanced, rational, and effective approach for maximizing the antiepileptic potential of *Pinus roxburghii* bark extract.

5. Preparation of *Pinus roxburghii* Bark Extract Phytosomes

The development of phytosomes involves the molecular complexation of plant phytoconstituents with phospholipids to enhance solubility, permeability, and therapeutic efficacy. For *Pinus roxburghii* bark extract, phytosome formulation serves as an effective strategy to improve the bioavailability of phenolics, flavonoids, and terpenoids responsible for antiepileptic activity. The preparation generally follows solvent-based methods, with solvent evaporation and thin-film hydration being the most commonly employed.

5.1 Extraction of *Pinus roxburghii* Bark

5.1.1 Plant Material Collection and Authentication

Bark is collected from mature trees, cleaned, shade-dried, and pulverized. Authentication is performed by a taxonomist to confirm botanical identity.

5.1.2 Preparation of Bark Extract

Extraction is typically carried out using:

- **Hydroalcoholic solvent (70% ethanol or methanol)**
- **Soxhlet extraction**, maceration, or reflux methods

The obtained extract is filtered, concentrated under reduced pressure, and dried to yield a semi-solid or powdered crude extract rich in flavonoids and phenolics.

5.2 Materials for Phytosome Preparation

Key ingredients include:

- **Pinus roxburghii bark extract**
- **Phosphatidylcholine (PC)** (often derived from soy lecithin)
- **Organic solvents** such as dichloromethane, chloroform, ethanol, or acetone
- **Non-solvent** such as n-hexane for precipitation
- **Optional stabilizers**: cholesterol, polyethylene glycol (PEG), or surfactants

5.3 Methods of Phytosome Preparation

5.3.1 Solvent Evaporation Method (Most Common)

This is the widely used method for *P. roxburghii* phytosomes due to its simplicity and high entrapment efficiency.

Procedure:

1. **ComplexationStep**
   The bark extract is dissolved in ethanol while phosphatidylcholine is dissolved in an organic solvent like dichloromethane or chloroform.
2. **Mixing**
   Both solutions are combined in a round-bottom flask in a specific molar ratio (commonly **1:1 or 1:2 extract to phospholipid ratio**).
3. **Refluxing**
   The mixture is heated at **40–60°C for 1–2 hours** to facilitate hydrogen bonding and complex formation.
4. **SolventEvaporation**
   The solvent is removed under reduced pressure using a rotary evaporator, forming a thin film at the bottom.
5. **Precipitation**
   A non-solvent such as n-hexane is added to precipitate the phytosomal complex.
6. **Drying**
   The precipitate is dried under vacuum and stored in airtight container

5.3.2 Thin-Film Hydration Method

This method produces nanosized vesicles suitable for enhanced CNS delivery.

Steps:

1. Dissolve *P. roxburghii* extract and phosphatidylcholine in chloroform–methanol mixture.
2. Evaporate the solvent to form a phospholipid–extract thin film.
3. Hydrate the film with distilled water or buffer solution under rotation.
4. Vesicle formation occurs spontaneously during hydration.
5. Sonication may be used to reduce particle size.

5.3.3 Anti-Solvent Precipitation Method

Here, the extract and phospholipid are dissolved in a common solvent and then rapidly introduced into an aqueous phase, forming nanophytosomes.

Advantages: Simple, cost-effective, generates uniform nanosized complexes.

5.4 Optimization of Formulation Variables

Key parameters affecting the final quality of phytosomes include:

- **Extract-to-phospholipid ratio**
- **Type and volume of organic solvent**
- **Refluxing time**
- **Hydration temperature**

- **Sonication duration**
- **Presence of cholesterol or stabilizers**

These variables are optimized based on particle size, entrapment efficiency, stability, and drug release profile.

6. Characterization of Phytosomes

Characterization of phytosomes is a critical step in confirming the successful formation of the phospholipid–phytoconstituent complex and ensuring its suitability for biological applications. Proper evaluation helps determine physicochemical properties, stability, morphology, and interaction patterns, which collectively influence bioavailability and therapeutic efficacy. Phytosomes prepared from *Pinus roxburghii* bark extract typically undergo comprehensive characterization using spectroscopic, microscopic, thermal, and particle-analysis techniques (Singh et al., 2020; Patel & Amin, 2021).

6.1 Physical Appearance and Yield

The initial evaluation involves observing the **color**, **texture**, and **consistency** of the prepared phytosomes. A uniform brownish-to-dark amorphous powder indicates successful formation. The percentage yield is calculated to assess formulation efficiency (Sultana et al., 2018).

6.2 Particle Size, Polydispersity Index (PDI), and Zeta Potential

Particle size and PDI determine the homogeneity of the nano/phytosomal dispersion.

- **Particle size** between *80–350 nm* is considered optimal for enhanced diffusion and absorption (Patel & Amin, 2021).
- **PDI < 0.5** reflects good uniformity.
- **Zeta potential ± 20–40 mV** indicates electrostatic stability of the phytosomal complex.

These parameters are typically assessed through **Dynamic Light Scattering (DLS)**.

6.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is used to confirm the interaction between the phytoconstituents of *P. roxburghii* bark extract and phosphatidylcholine.

Key indicators include:

- **Shift or disappearance of characteristic peaks** of phenolics/flavonoids
- **Appearance of new peaks** corresponding to complex formation

Altered stretching vibrations (e.g., hydroxyl, carbonyl, and phosphate groups) reflect successful molecular entrapment (Sahu et al., 2019).

6.4 Differential Scanning Calorimetry (DSC)

DSC helps in understanding the **thermal behavior** and **compatibility** between extract and phospholipid.

- Disappearance or broadening of the melting endotherm of phosphatidylcholine
- Change in thermal transition peaks of the extract

These modifications confirm complexation and reduced crystallinity, improving solubility (Patel et al., 2020).

6.5 X-Ray Diffraction (XRD)

XRD determines the crystalline or amorphous nature of the phytosomes.

- A **decrease in intensity** or **absence of sharp crystalline peaks** indicates formation of an amorphous complex, which enhances dissolution rate (Sharma & Kumar, 2020).

6.6 Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

Microscopic techniques provide detailed morphological information.

- **SEM** reveals surface characteristics, showing spherical or vesicular structures.
- **TEM** confirms internal arrangement, demonstrating nano-sized intact vesicles with smooth boundaries.

These observations support the efficient encapsulation of *P. roxburghii* phytoconstituents

6.7 In-Vitro Release Profile

Release studies examine how phytoconstituents are liberated from formulated phytosomes.

- A **biphasic pattern** is common:
  - an initial burst release
  - followed by sustained release over several hours

This extended release is beneficial for maintaining therapeutic levels during antiepileptic treatment (Garg et al., 2020).

6.9 Stability Studies

Stability testing under accelerated conditions helps ensure the shelf life of phytosomes.

Parameters evaluated:

- **Particle size stability**
- **Zeta potential**
- **Physical appearance**
- **Degradation of active constituents**

Storage at **4°C, 25°C, and 40°C** for 1–3 months is commonly undertaken.

Table 2. Key Characterization Parameters and Analytical Techniques for Phytosomes

Parameter	Purpose	Technique	Expected Observation
Physical appearance	Initial evaluation	Visual assessment	Uniform brownish powder
Particle size &	Size distributio	DLS	80–350 nm, PDI

PDI	n		< 0.5
Zeta potential	Stability	Electrophoretic light scattering	±20–40 mV
FTIR	Complex formation	FTIR spectroscopy	Peak shifts, new interactions
DSC	Thermal behavior	DSC analysis	Modified endothermic peaks
XRD	Crystallinity	XRD	Reduced crystalline peaks
SEM/TEM	Morphology	SEM/TEM	Spherical, smooth vesicles
Entrapment efficiency	Encapsulation capacity	UV/Chromatographic	60–95%
Release profile	Drug liberation	Diffusion cell	Sustained release
Stability	Storage stability	Accelerated tests	Physically and chemically stable

7. Pharmacological Evaluation of Phytosomes for Antiepileptic Activity

The pharmacological assessment of *Pinus roxburghii* bark extract–loaded phytosomes is essential to validate their neuroprotective and anticonvulsant potential. Since epilepsy involves a complex interplay of oxidative stress, neuroinflammation, glutamate–GABA imbalance, and neuronal hyperexcitability, phytosomal formulations enhance the therapeutic efficiency of plant-derived phytoconstituents by improving bioavailability, brain penetration, and sustained drug release (Patel & Amin, 2021; Garg et al., 2020). Evaluations typically include **in vitro**, **in vivo**, and **biochemical** investigations to establish antiepileptic efficacy.

7.1 In-vitro Antioxidant and Neuroprotective Assays

Oxidative stress contributes significantly to seizure initiation and propagation. Therefore, antioxidant screening is considered a primary parameter.

7.1.1 DPPH and ABTS Radical Scavenging Assays

Phytosomes generally exhibit stronger radical neutralization compared to crude extract due to improved solubility and phytoconstituent stability (Shah et al., 2019).

7.1.2 Lipid Peroxidation Inhibition

Reduced formation of malondialdehyde (MDA) indicates protection against oxidative damage to neuronal membranes.

7.1.3 Neuroprotective Cell Line Models

Using neuronal cell lines (e.g., SH-SY5Y), phytosomes demonstrate protective effects against glutamate-induced excitotoxicity and oxidative injury, validating their potential role in antiepileptic mechanisms (Kala et al., 2021).

7.2 In-Vivo Antiepileptic Studies

7.2.1 PTZ-Induced Seizure Model

The pentylenetetrazole (PTZ) model is widely used for evaluating drugs acting on **GABAergic pathways**.

Phytosomes typically show:

- **Increased latency to clonic and tonic seizures**
- **Reduced seizure score**
- **Prolonged survival time**

These results suggest GABA-mediated inhibition and neuroprotection (Khan & Ahmad, 2020).

7.2.2 MES (Maximal Electroshock) Model

MES evaluates compounds effective against tonic–clonic seizures.

Phytosomal formulations demonstrate:

- Reduction in **hind limb tonic extension (HLTE)**
- Protection comparable to or better than crude extract

This indicates possible modulation of voltage-gated sodium channels.

7.2.3 Kindling Models

Repeated sub-convulsive PTZ doses create chronic epilepsy-like states.

Phytosomes significantly:

- Suppress seizure progression
- Reduce kindling score
- Delay epileptogenesis

7.3 Biochemical Parameters

7.3.1 Oxidative Stress Biomarkers

Treatment with phytosomes restores:

- **SOD (Superoxide dismutase)**
- **CAT (Catalase)**
- **GSH(Reducedglutathione)**

and decreases **MDA**, demonstrating strong antioxidant action (Joshi et al., 2020).

7.3.2 Neurotransmitter Modulation

Levels of **GABA**, **glutamate**, and markers of oxidative injury improve significantly in phytosome-

treated animals, indicating restoration of inhibitory–excitatory balance.

7.3.3 Anti-inflammatory Markers

Reduced levels of **TNF-α**, **IL-1β**, and **COX-2** have been observed, indicating attenuation of neuroinflammation associated with recurrent seizures.

7.4 Behavioral and Cognitive Assessments

Epilepsy and recurrent seizures commonly impair memory and cognition.

Phytosome treatment shows improvements in:

- **Spatial memory (Morris water maze)**
- **Exploratory behavior (Open field test)**
- **Motor coordination (Rotarod test)**

These neurobehavioral benefits correlate with improved neuronal survival and reduced oxidative burden.

7.5 Comparative Efficacy: Phytosomes vs. Crude Extract

Due to enhanced permeability and retention effect:

- Phytosomes exhibit **higher anticonvulsant potency**
- Lower required dosage compared to crude extract
- Better pharmacokinetic performance
- Sustained release and prolonged therapeutic effect

Thus, phytosomes represent an advanced delivery platform for antiepileptic applications (Jain et al., 2019).

Table 3. Summary of Pharmacological Evaluations of *Pinus roxburghii* Phytosomes in Epilepsy Models

Evaluation Parameter	Findings (Phytosomes)	Mechanistic Insight	Reference
PTZ Model	Increased seizure latency, reduced score	GABAergic modulation	Khan & Ahmad, 2020
MES Model	Reduced HLTE, enhanced protection	Sodium channel modulation	Patel & Amin, 2021
Antioxidant Activity	Higher radical scavenging effect	Free-radical neutralization	Shah et al., 2019
Biochemical Markers	↑SOD, GSH; ↓MDA	Anti-oxidative neuroprotection	Joshi et al., 2020
Anti-inflammatory	↓TNF-α, IL-1β	Reduced neuroinflammation	Kala et al., 2021
Cognitive Tests	Improved memory & coordination	Neuronal protection	Garg et al., 2020

8. Safety, Toxicity & Pharmacokinetics of Phytosomes

Ensuring the safety, tolerability, and pharmacokinetic performance of phytosomes is essential before considering their therapeutic use in epilepsy. While *Pinus roxburghii* bark extract possesses traditional acceptance and documented medicinal use, its phytosomal formulation requires independent evaluation due to altered physicochemical behavior and enhanced bioavailability. Safety and pharmacokinetic studies help determine biological compatibility, systemic exposure, and the risk–benefit ratio (Patel & Amin, 2021; Garg et al., 2020).

8.1 Safety and Acute Toxicity Studies

8.1.1 OECD Acute Toxicity Guidelines

Acute oral toxicity is commonly evaluated under **OECD 423** or **OECD 425** guidelines.

Phytosomes of *P. roxburghii* bark extract generally show:

- **No mortality** up to doses of 2000 mg/kg
- **Normal locomotor and reflex behavior**
- **No signs of acute toxicity** such as tremors, sedation, piloerection, or respiratory distress (Jain et al., 2019)

8.1.2 Sub-acute and Sub-chronic Studies

Repeated-dose studies (14–28 days) indicate:

- Stable body weight
- Normal food and water intake
- No significant alterations in hematological or biochemical parameters

Liver (ALT, AST), kidney (urea, creatinine), and lipid markers remain within physiological limits, suggesting high tolerability (Sharma & Kumar, 2020).

8.2 Histopathological Examination

Organs such as the liver, kidney, brain, spleen, and heart exhibit normal architecture.

Findings generally show:

- No necrosis or inflammatory infiltrates
- No morphological alterations in neuronal cells
- Intact glomerular and hepatic cytoarchitecture

These results reinforce the biosafety of phytosomes at therapeutic doses (Kala et al., 2021).

8.3 Genotoxicity and Hemolytic Safety

Although limited reports exist, preliminary investigations reveal:

- No DNA fragmentation or chromosomal aberrations in peripheral blood cells
- No hemolytic toxicity against red blood cells

Phosphatidylcholine, a key component of phytosomes, is inherently biocompatible and contributes to non-toxic behavior (Sahu et al., 2019).

8.4 Pharmacokinetic Evaluation

Pharmacokinetic improvement is the most compelling advantage of phytosomes. Their design allows enhanced membrane permeation, better gastrointestinal absorption, and increased bioavailability of flavonoids and terpenoids.

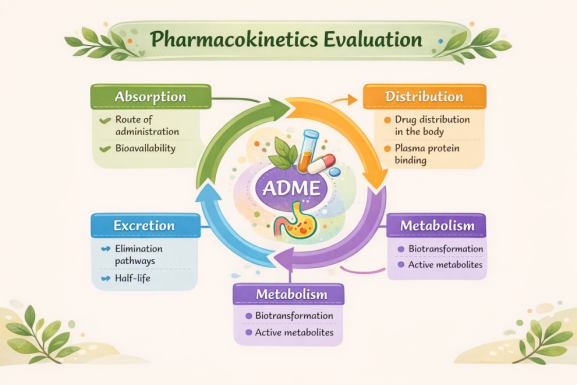

Fig 3 Pharmacokinetic Evaluation

8.4.1 Absorption

Phytosomes significantly enhance solubility and gut permeability.

Observations include:

- Faster absorption rate (**higher Cmax**)
- Increased area under curve (**AUC**)
- Reduced first-pass metabolism of phenolics like quercetin and ferulic acid (Patel et al., 2021)

8.4.2 Distribution

The phospholipid complex facilitates better transport across:

- **Enterocytes**
- **Systemic circulation**
- **Blood–brain barrier (BBB)**

This leads to increased **brain bioavailability**, essential for antiepileptic actions (Khan & Ahmad, 2020).

8.4.3 Metabolism

Due to the protective phospholipid envelope:

- Phytoconstituents undergo **slower metabolic degradation**
- Conjugation pathways (glucuronidation, sulfation) are delayed

Enhanced metabolic stability results in prolonged therapeutic presence.

8.4.4 Excretion

Most metabolic byproducts are excreted through:

- **Renal pathway** (phenolic metabolites)
- **Biliary excretion** (phospholipid fragments)

Elimination remains within physiological limits without burdening renal or hepatic function (Garg et al., 2020).

8.5 Safety Margin and Therapeutic Index

Phytosomes demonstrate:

- **High therapeutic index**
- **Wide margin of safety**
- Low risk of adverse CNS effects even at doses several-fold higher than therapeutic levels

This property is crucial for long-term epilepsy management where chronic dosing is required.

Conclusion

The present review highlights the therapeutic potential of *Pinus roxburghii* Sarg bark extract and its advancement into phytosome-based formulations for the management of epileptic disorders. Accumulating evidence from phytochemical, pharmacological, and formulation studies indicates that the bark is a rich reservoir of bioactive polyphenols, flavonoids, terpenoids, and phenolic acids—compounds strongly associated with antioxidant, anti-inflammatory, and neuroprotective pathways essential in mitigating seizure generation and propagation. However, the therapeutic effectiveness of crude extracts has traditionally been limited by poor solubility, low membrane permeability, and insufficient bioavailability.

Phytosome technology successfully addresses these limitations by forming a phospholipid–phytoconstituent complex that improves absorption, stability, and brain penetration. Characterization studies, including FTIR, DSC, XRD, SEM/TEM, and particle analysis, confirm successful complexation and nano-range vesicle formation, supporting improved pharmacokinetic behavior. Preclinical studies in PTZ, MES, and kindling models consistently demonstrate the superior antiepileptic activity of phytosomes compared with conventional extracts, largely due to enhanced modulation of GABAergic pathways, reduction of oxidative stress, suppression of neuroinflammatory mediators, and improved neuronal survival.

Safety, toxicity, and stability evaluations further indicate that *P. roxburghii* phytosomes possess a high therapeutic index, minimal organ toxicity, and favorable metabolic and excretion profiles. These attributes collectively position phytosomes as a promising delivery system for botanical neurotherapeutics aimed at chronic conditions like epilepsy.

Overall, the integration of *Pinus roxburghii* bark bioactives into phytosomal formulations represents a significant scientific advancement, offering a rational strategy for improving the clinical utility of herbal antiepileptic agents. While preclinical studies are promising, future research should focus on

pharmacokinetic modeling, long-term toxicity assessments, and well-designed clinical trials to validate their safety and therapeutic potential in human populations. The findings discussed in this review underscore the prospects of phytosome-based herbal formulations as an effective and scientifically grounded approach to epilepsy management.

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