

Pharmacogenetic & Physicochemical evaluation of *Betula utilis* and *Annona squamosa*

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Abstract

Background: *Betula utilis* and *Annona squamosa* are some indigenous medicinal plants that have served as traditional medicine over the centuries. **Aim:** The present study therefore evaluated pharmacogenetic and physiochemical properties of these plants. Pharmacogenetic evaluation showed that both plants have distinct genetic characteristics, which significantly modify their medicinal properties. For physiochemical evaluation, *Betula utilis* contains betulinic acid, lupeol, and other triterpenoids, while *Annona squamosa* contains saponins, cardiac glycosides, flavonoids, terpenoids, phenolics, and sterols. **Methods:** Collection and Authentication of *Betula utilis* and *Annona squamosa*, pharmacogenetic studies, and physicochemical properties were evaluated. **Conclusion:** Different antioxidant assay systems, and antimutagenic activities in both plants, showed that *Annona squamosa* presented remarkable antioxidant and antimutagenic activities. This research provides an extensive description of pharmacogenetic & physiochemical features about *Betula utilis* and *Annona squamosa*, useful in the development of new drugs and therapies. **Major Findings:** The pharmacognostic evaluation established essential standards for the identification and verification of these plants, and using phytochemical screening, it was discovered that chemicals with bioactivity, particularly phenolics and bioflavonoids, known for their antioxidant properties.

Keywords: *Betula utilis*, *Annona squamosa*, Pharmacogenetic, Physiochemical, Extraction,

How to cite this article: Pragti Singh¹ Dr. Pavan Kumar². Pharmacogenetic & Physicochemical evaluation of *Betula utilis* and *Annona squamosa*. Int J Drug Deliv Technol. 2026;16(84s):2019-2030. DOI: 10.25258/ijddt.16.84s.204

Introduction

According to WHO estimates, more than 70,000 plant species have been used medicinally, and more than 80% of the world's population uses traditional medicine to avoid illnesses.

The Ayurvedic medical system in India is extremely important for the treatment of a variety of disorders because of its major contributions to the creation of herbal plants. People have utilised traditional medicine as a home cure in addition to Ayurveda. Since the COVID-19 pandemic, herbal remedies have garnered attention due to their proven efficacy in immunomodulatory therapy and prevention. There is currently a great drive to do comprehensive, empirically supported studies on the therapeutic qualities of Ayurvedic medications and medicinal plants¹. According to Indian traditional medicine, one of the most valued wood species is *Betula utilis*. Tridosha "Vata", "Pitta", and "Kapha" therapy are all included. Medicinal preparations include infusion, powder, paste, and decoction to treat other types of illness. Other uses include food, gums, and resins that come from the plant Rheumatism, fractured bones, tight joints, swelling, asthma, and blood purification are all treated with the bark, leaves, and resin. "Kidney and bladder stones," "urinary tract infections," and "a leaf decoction of *B. utilis*" are treated using the leaves²⁻⁴. India is essentially a global herbarium. Due to our abundance of plants and herbs, we in India use them as our primary source of medicine. Herbs that contribute to our diet and offer us extra medicinal

benefits are in high demand; one such plant is *Betula utilis*, known as Bhoj Patra. A significant medicinal plant, *Betula utilis* is a member of the Betulaceae family. The Himalayas are home to a large population of it. It has served a traditional purpose in medicine for hundreds of years, taking in any kind of trouble, be it fever, rheumatism, or skin diseases. The plant is loaded with bioactive compounds, e.g., betulinic acid, lupeol, and others, mostly belonging to the triterpenoid family⁵⁻⁸.

The *Annona* is one of the most important plants in the Annonaceae family⁹. The common names for this plant are in Hindi, sharifa means "custard apple" and in Telugu, sitaphalam

¹⁰. Throughout India, the little, evergreen tree *Annona squamosa* is grown for its fruits. In traditional medicine, different parts of the tree are utilised to treat various ailments¹¹.

Both synthetic and conventional herbal medicines are derived from natural ingredients. In several regions of the world, they continue to serve as the main healthcare system. Numerous actions have been Antimutagenic, Anthelmintic, Scavenging, Antimicrobial, Antidiabetic, Lcidal, Hepatoprotective, Antithyroid, Antigenotoxic, Anti-plasmodial, and Molluscicidal are among the compounds that have been investigated on the *Annona squamosa* plant. In addition, many plant components have been utilised in traditional medicine; the roots are purgative and helpful for mental depression; the fruits are cooling and haematinic. Bark as atonics; leaves—crushed leaves

are applied to wounds and ulcers; a leaf decoction is administered in instances of diarrhoea; seeds as an abortifacient, helpful in eliminating lice in the hair; sedative, vomiting, coughing, malignant tumour, and fortifying muscles^{12,13}. Typically, a single fruit contains 30–40 dark brown to black seeds of *A. squamosa*. One plant species with 2n-14 is categorised as diploid, *A. squamosa*¹⁴.

Betula utilis and *Annona squamosa* have been traditionally used in the treatment of health problems for several centuries. Himalayan birch is a Latin name for Himalayan birch. This seasonal tree, formerly known as the sugar apple, spreads widely throughout the Himalayas. All parts of such plants have been documented as having multiple medicinal uses including reduced inflammation, antimicrobial, & antioxidant properties. Still, the pharmacogenetic and physicochemical properties of these plants are not well recorded¹⁵⁻¹⁷. While pharmacogenetics is understood as studying how genetic variabilities affect an individual's response to drugs, in herbal medicine pharmacogenetics can help to identify the genetic prerequisite responsible for the efficacy and safety of phytotherapies. Conversely, physicochemical characterization analyzes the physical and chemical properties of plant extracts¹⁸. This study was designed to assess the pharmacogenetics and physicochemical characteristics of *Annona squamosa* and *Betula utilis*. To elicit the genetic variations of the plants, we employed a variety of molecular biology techniques¹⁹. Furthermore, the chemical compounds of the plant were analyzed by converting the physicochemical analysis into HPLC (high-performance liquid chromatography) and other techniques²⁰⁻²⁵.

1 METHODS & MATERIALS

1.1 *Annona squamosa* and *Betula utilis* collection and authentication

1.1.1 *Betula utilis*

The collected leaves of *Betula utilis* belong to BHU, Varanasi. A voucher specimen of each plant was deposited for future reference. *Betula utilis* leaves (Ref. No. *Betula*. 2023/1).

1.1.2 *Annona squamosa*

The roots of *Annona squamosa* were collected from BHU (Banaras Hindu University), Varanasi. A voucher specimen of each plant was deposited for future reference. *Annona squamosa* roots (Ref. No. *Annona*. 2023/1).

1.2 Reagents and Chemicals

The leaves of *Betula utilis* and roots of *Annona squamosa* (collected from BHU), potassium hydroxide, chloral hydrate, hydrochloric acid, petroleum ether, and methanol. Hager's, Mayer's, Fehling's, Barfoed's, Molisch, Benedict's, Ninhydrin solutions, Alkaline solution, Zinc chloride, Ferric chloride, and Millon's reagent (Span Diagnostics Ltd., India, Sigma Chemicals, USA, Sd.

fine chemicals, Mumbai)²⁶⁻³².

1.3 Pharmacogenetics studies

1.3.1 Analysis of morphology

The fresh leaves and roots of both plant species have been used as morphological observations. The macromorphological capabilities of leaves and roots have been discovered beneath the magnifying lens³³.

1.3.2 Microscopical Evaluation

Photographs were taken and the specimen's morphological characteristics (bark and leaves) were examined. The transverse segment (TS) replaced the longitudinal phase as the favoured method for microscopic study. The high-quality leaf parts were hand-chopped. The pieces were treated with 5% potassium hydroxide and 20% chloral hydrate to remove the plant's chlorophyll and other colours. The Olympus Microscope, version Olympus (India), linked to the YOKO CCD digital camera was the subject of serious images at different magnifications³⁴.

1.3.3 Extraction

The extraction process was carried out using analytical-grade solvents in accordance with standard procedure³⁵. Solvents that were appropriate for the characteristics of secondary metabolites were used in successive solvent extraction. Petroleum ether, methanol, and water (non-polar to polar) were used in succession using the Soxhlet extractor to separate the coarse powder from the dried plant material.

1.3.4 Evaluation of physicochemical

According to WHO guidelines, a number of physicochemical parameters were measured, including total ash, acid insoluble ash values, water-soluble ash values, extractive values, moisture content, and foreign particles³⁶.

1.3.5 Determining the drying loss

The two grammes of dried crude powder were put in an evaporating dish and burned at 105 degrees Celsius until they reached a consistent weight. Following the drying process, the weight was recorded and computed. The original weight was used to compute the percentage of loss during drying.

1.3.6 Determination of total ash

A silica crucible was used to heat two grams of the dried powder from the crude drug, with the temperature being gradually increased to 450 °C until the powder became white, indicating that carbon was no longer present. After cooling the ash in desiccators, it was weighed immediately. The air-dried material's total ash concentration was determined in milligrams per gram.

1.3.7 Acid-insoluble ash determination

A 25 ml volume of hydrochloric acid (70 g/L) was added to the crucible holding the whole ash. After setting a watch glass on top, the fluid was slowly brought to a boil for five minutes. After that, five millilitres of hot water were used to rinse the watch glass before it was put back in the crucible. The

insoluble substance was captured using ash-free filter paper, which was then neutralised by washing it in hot water. The insoluble material-containing filter paper was put back into the original crucible, dried on a hot plate, and heated until the weight remained constant. After 30 minutes of cooling in a desiccator, the residue was weighed right away. The mg/g of air-dried material was used to quantify the acid-insoluble ash.

1.3.8 Water-soluble ash measurement

After adding 25 millilitres of water to the crucible containing the entire ash, it boiled for five minutes. Using ashless filter paper, the insoluble material was gathered. It was cooked in a crucible for fifteen minutes after being washed with hot water. From the weight of insoluble elements, the weight of total ash was subtracted. Milligrammes per gramme were used to quantify the amount of water-soluble ash in the air-dried material.

1.3.9 Determination of petroleum ether soluble extractive value

A conical flask containing five grams of dried crude drug powder and 100 millilitres of petroleum ether was sealed with cotton wool, plugged & in a rotating shaker set to one hundred twenty beats per minute for twenty-four hrs. After filtering it, the filtrate was dried by evaporating it at 105°C until the weight remained constant. Regarding the original sample, the extractable matter % was estimated.

1.3.10 Determination of methanol soluble extractive value

A conical flask containing five grams of dried crude drug powder and 100 millilitres of methanol, cotton wool plugged & in a rotating shaker set to one hundred twenty beats per minute for twenty-four hrs. After filtering it, the filtrate was dried by evaporating it at 105°C until the weight remained constant. Regarding the original sample, the percentage of the extractable materials was calculated.

1.3.11 Water-soluble extractive value analysis

A flask that is conical containing five grams of dried crude drug powder and 100 millilitres of water, cotton wool plugged & placed in a rotating shaker set to one hundred twenty beats per minute. After filtering it, the filtrate remained dried by evaporating it at 105°C until the weight remained constant. Regarding the original sample, the percentage of the extractable materials was calculated.

1.3.12 Solubility

The methanolic extract's quantitative solubility test was conducted in several solvents. To evaluate the extract's solubility in various solvents, five milligrams were weighed. Each solvent was filled with the extract until it was saturated. In mg/ml, the solubility was calculated.

1.4 Plant Extract Preparation

The 2.5 kg of collected plant material was air dried, then dried in a tray dryer under ideal conditions, and finally ground into powder. The 1.2 kg of powdered

crude medicine was extracted using a Soxhlet extractor with PE, methanol, and water. Further filtering and lowered pressure at 40°C produced a syrupy mass from the crude solution, which was then stored in a desiccator.

1.5 HPTLC Analysis

HPTLC is advanced analytical techniques used for purification, isolation, power, reproducibility advanced to the simple TLC. According to the utilization HPTLC micro plates with fine particle sizes and precise sophisticated machine for every step of the chromatographic method (sample injection, chromatogram development and its analysis), thus HPTLC important tool to provides the qualitative and quantitative fingerprinting profile of given sample with reference to standard marker compound.

1.5.1 Procedure: HPTLC analysis of Methanolic extract of both plants

HPTLC system equipped (CAMAG) plus 5 Linomat applicator (Camag Linomat 5 "Linomat5_130439" S/N 130439 (1.00.12), TLC scanner 3 (CAMAG TLC Scanner 3 "Scanner3_130520" S/N 130520 (1.15.26), with illuminating instrument reprostar 3 (CAMAG Reprostar 3 : 130516 (Repro3_130516) with Digital camera type: snr en lens (DXA252 : 275584707, Computar, 16 mm, f5.0). for visual detection, operated by software WinCATS were used. All the analytical grade manstrum required for HPTLC fingerprinting technique was procured by Merck. About 100 mg BVE were mixed in 5ml CH₃OH for HPTLC fingerprinting evaluation served as test sample. The solute (4, 8, 10 µl) spotted in the HPTLC silica gel 60F-255 plate with length of 6.0 mm with the help of Camag microliter syringe. Linear ascending chromatogram was development to achieve good resolution of phytochemicals using glass chamber saturated with both the solvent system with the same chromatographic column. The chromatographic plate was placed at fluorescence cabinet and taken a picture under normal light, and UV visible light at 254 and 366nm respectively. 3D analysis was analysed on CAMAG-TLC scanner3& governed by operating system WinCATS (V 1.15.26 Camag). The HPTLC fingerprinting evaluation of the *Betula utilis* leaves (BUMX) and *Annona squamosa* roots (ASME) was performed at NBRI, Lucknow.

Table 1 Parameters used in HPTLC analysis of Methanolic extract of *Betula utilis* leaves (BUMX) and *Annona squamosa* roots (ASME)

Plant name:	<i>Betula utilis</i> leaves (BUMX)	<i>Annona squamosa</i> roots (ASME)
Used plant	Leaves	Roots

part:		
Sample spotting:		
1st Track	BUMX	ASME
2nd Track	BUMX	ASME
3rd Track	BUMX	ASME
TLC Band Length	6.0 mm	6.0 mm
TLC plate size	10.0 x 5.0 centimeters	10.0 x 5.0 centimeters
Two-band distance	15 mm	15.0 mm
Sample conc	20 mg/ml	20 mg/ml
Total Sample vol	4, 8, 10 µl	4, 8, 10 µl
MP	Toluene: EA: FA (5:4:1)	n-hexane: EA: formic acid: GAA (7:3:0.1:0.1)
Slit dimension	6.0x0.30mm, Micro	6.00x0.30 mm

2.6 Analytical statistics

The mean±SD of separate experiments is used to express data. Using Graph Pad Prism software (Version 5.01), one-way ANOVA and Dunnett's Multiple Comparison Test were used to compare the means. A p-value of less than 0.05 is regarded as statistically significant.

2 Results

2.1 Collection and Authentication of *Betula utilis* and *Annona squamosa*

2.1.1 *Betula utilis*

The collected leaves of *Betula utilis* belong to BHU, Varanasi. A voucher specimen of each plant was deposited for future reference. *Betula utilis* leaves (Ref. No. *Betula*. 2023/1).

2.1.2 *Annona squamosa*

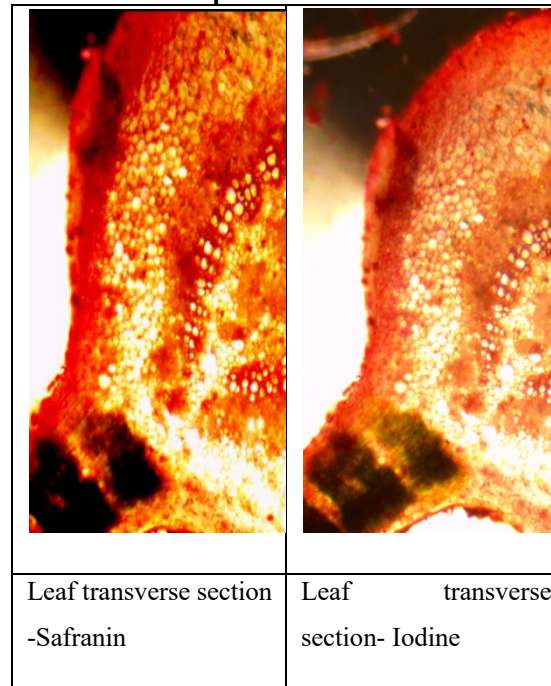
The roots of *Annona squamosa* were collected from BHU (Banaras Hindu University), Varanasi. A voucher specimen of each plant was deposited for future reference. *Annona squamosa* roots (Ref. No. *Annona*. 2023/1).

2.2 Macroscopical study

Table 2 Morphological characteristics of *Betula utilis* leaves and *Annona squamosa* roots

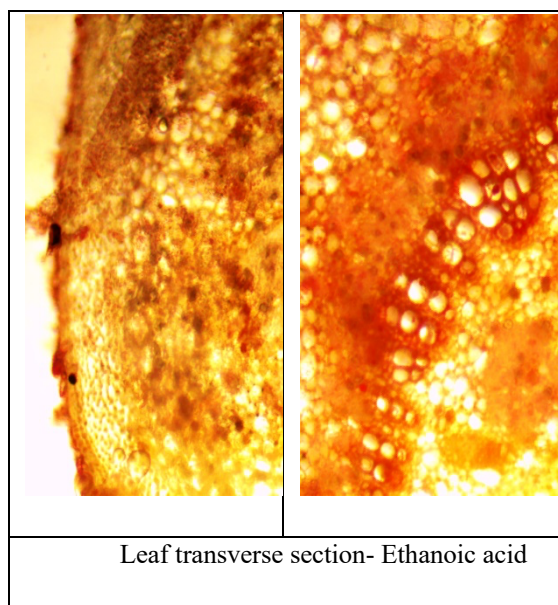
Parameters	Description	
	<i>Betula utilis</i> leaves	<i>Annona squamosa</i> roots
Colour	Green	Reddish Brown
Odour	Odourless	Odourless
Flavour	Mucilaginous	Characteristics
Dimensions	2.5-6 by 1.5-5 centimetres	Varying
Appearance	Silky	Rough
Nervation	Reticulatory	NA
Tip	Obtususe	NA
Lamina's shape	Green that is thin and glossy	NA
The margin	Dentate	---
Base	Rounded	---

2.2.1 Microscopical evaluation



Leaf transverse section -Safranin

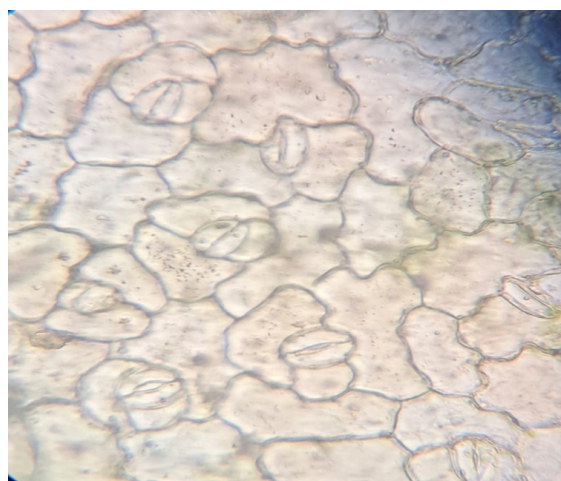
Leaf transverse section- Iodine

Figure 1 *Betula utilis* leaves' Transverse section

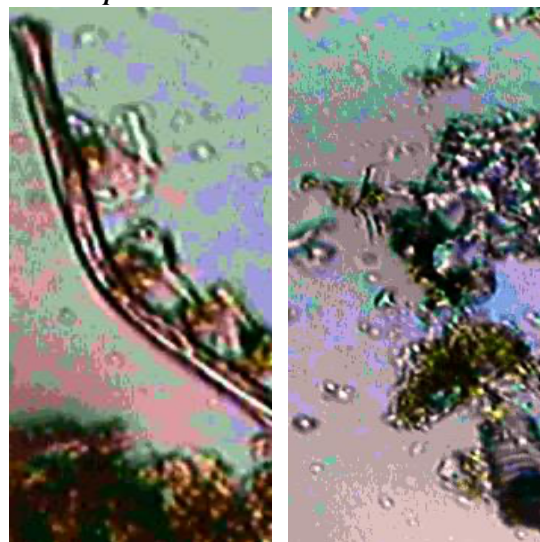
3.3 Quantitative microscopical evaluation

Table 3 Quantitative microscopical analysis of *Betula utilis* leaves

Particulars	<i>Betula utilis</i> leaves
Stomatal number upper epidermis	04 -07
Lower epidermis stomatal count	03 - 05
I (sto. Ind.)	04 -06
Vein -islets no.	08 - 11
Number of vein terminations	05 - 07
Ratio of palisades	06 - 08

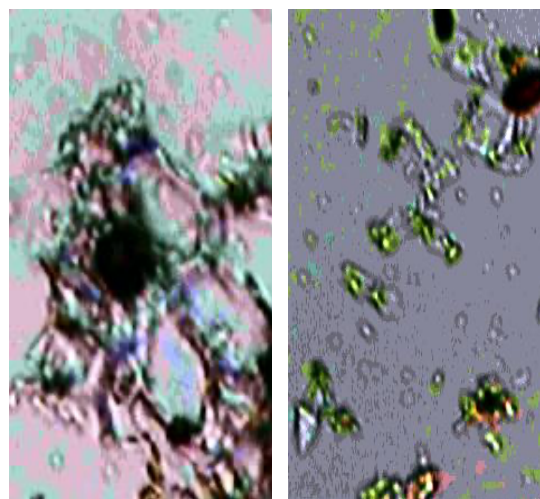
Figure 2 Anisocytic Stomata of *Betula utilis* leaves

2.2.2 Microscopical evaluation of *Annona squamosa* roots



Multicellular
Trichomes

Xylem Vessels &
Latex Vessels



Parenchymatous
tissues

Calcium Oxalate
Crystals

Fig. 3 *Annona squamosa* root powder microscopy

2.3 Physical-chemical examination of *Annona squamosa* roots and *Betula utilis* leaves

2.3.1 Physical-chemical characteristics

Table 6.3 presents the results of the aforementioned study of powdered *Annona squamosa* roots and *Betula utilis* leaves. The results for each parameter are shown as a percentage of the shade-dried plant specimen's age.

Table. 4 Physical-chemical examination of *Annona squamosa* roots and *Betula utilis* leaves

S. No.	Particulars	Unit	<i>Betula utilis</i> leaves	<i>Annona squamosa</i> roots
			% (% w/w)	% (% w/w)
	Loss on Drying	% w/w	3.90	2.40
	Total Ash Content	% w/w	5.90	4.10
	Water Soluble Ash	% w/w	4.50	3.20
	Acid Insoluble Ash	% w/w	0.90	1.20
	Sulphated Ash	% w/w	2.20	1.60
	Hexane Extractive Value	% w/w	1.50	1.40
	Methanol Extractive Value	% w/w	2.00	2.20
	Aqueous Extractive Value	% w/w	5.10	6.20

2.4 Phytochemical Screening

2.4.1 Chemical tests of *Betula utilis* leaves**Table 5 Phytochemical test of various extracts of *Betula utilis* leaves**

Metabolites	PE	Methanol	Aqueous extract
Alkaloids			
Hager's Test	ND	(+)	(++)
Mayer's Test	ND	ND	(+)
Carbohydrate test			
Fehling's Test	(+)	(++)	ND
Barfoed's Test	ND		(+)
Molisch's Test	(+)	(++)	ND
Benedict's Test	(+)	ND	(+)
Fixed oils and			

fats			
Grease spot test	(++)	ND	(+)
Proteins & amino acids			
Biuret's Test	(+)	(+)	ND
ninhydrin Test	ND	ND	(+)
Millon's Test	(+)	(+)	(+)
Flavonoids			
Ferric chloride test	ND	(++)	(+++)
alkaline reagent test	(+)	ND	(+++)
Lead acetate test	(+)	ND	(++)
Zinc/ HCL reduction test	ND	ND	(++)
Shinoda's test	(+)	(+)	(++)
Steroids			
Salkowski tests	(++)	ND	ND
Saponins			
Foam tests	ND	(+)	(++)
Non- reducing carbohydrates			
Iodine test	ND	(++)	
Tannic acid test	ND	ND	(++)
Tannins & phenolic compounds			
Ferric chloride test	(+)	ND	(++)

Notation: (+): - Present, (++):- moderately present, (+++): - strongly present, ND:- not detected

2.4.2 Phytochemical tests of *Annona squamosa* roots**Table 6 Phytochemical test of various extracts of *Annona squamosa* roots**

Phytoconstituents class	PE	Methanol	Aqueous extract
Alkaloids			
Hager's test	ND	(++)	(+++)
Mayer's test	ND		(++)

Carbohydrates			
Fehling's test	(+)	(+)	ND
Barfoed's test	ND	ND	(+++)
Molisch's test	ND	ND	(+)
Benedict's test	(+)	(+)	(+)
Fixed oils and fats			
Filter paper test	(+)	ND	(+)
Amino Acids and Peptide			
Biuret's test	(+)	ND	ND
Ninhydrin's test	(+)	(++)	(+)
Millon's test	ND	(+)	(+)
Flavonoids			
Ferric chloride test	ND	(++)	(+++)
Alkaline reagent test	ND	(++)	(++)
Lead acetate test	ND	(++)	(++)
Zinc hydrochloride reduction test	(+)	(+)	(+++)
Shinoda test	ND	(++)	(+++)
Steroids			
Salkowski test	(++)	ND	ND
Saponins			
Froth test	ND	ND	(++)
Non-reducing carbohydrate			
Iodine Test	ND	(+)	
Tannins test	ND	(+)	(++)
Polyphenols			
Ferric chloride test	ND	(++)	(+++)

Notation: (+): - Present, (++) : - moderately present, (+++) : - strongly present, ND: - not detected

2.5 Fluorescence analysis

Table 7 Fluorescences Characteristics of Powder *Betula utilis* Leaf Powder

S. No.	Sample	Fluorescence under UV Light (364 nm)	Appearance under Visible Light	Fluorescence under Short-
--------	--------	--------------------------------------	--------------------------------	---------------------------

				Wave UV
	Untreated leaf Powder	Buff Green	Light Green	Light Yellow
	powder treated with concentrated nitric acid	Light green	Yellowish green	Pale Brown
	powder treated with concentrated sulphuric acid	Dark green	Black	light brown
	powder treated with 5% iodine solution	Dark green	dark brown	Buff Colour
	powder treated with N sodium hydroxide (aqueous)	Light brown	green	dark brown
	Powder treated with 1 N sodium hydroxide (methanolic)	green	Dark Green	Light Green
	Powder treated with 5%	Light Green	Yellowish Green	dark green

	ferric chloride			
	50% alcoholic extract	Green	Dark green	Purple
	Aqua. extract	Yellowish Green	Light Green	dark green

Table. 8. Powder fluorescence analysis Roots of *Annona squamosa*

Serial Number	Treatment with powder	UV at a length of 364 nm	In normal light	Light with a short wave
1)	POWDER	light brown	brown	purplish brown
2)	powder treated with concentrated nitric acid	light red	yellowish brown	blood red
3)	powder treated with concentrated sulphuric acid	dark brown	black	brown
4)	powder treated with 5% iodine solution	brown	dark brown	yellowish brown
5)	powder treated	red brown	red green	green

	with 1 N sodium hydroxide (aqueous)			
6)	powder treated with N sodium hydroxide (methanolic)	yellowish brown	yellowish green	buff color
7)	Powder + 5% FeCl ₃	Light green	Yellowish green	Dark Green
8)	50% Alcoholic extract	yellowish Brown	Dark Brown	Light yellow
9)	Aqueous extract	light yellow to brown	yellowish brown	dark yellow

2.6 HPTLC Quantitative Fingerprinting Analysis

2.6.1 Methanolic extract of *Betula utilis* leaves

HPTLC is now the simplest and fastest separation method available, providing the highest level of accuracy and precision while also creating a wide range of application opportunities. *Betula utilis* leaves displayed seven peaks in the 200–800 nm spectral range. The results show that the R_f values are 0.11, 0.12, 0.15, 0.19, 0.52, 0.58, 0.67, 0.69, 0.71, 0.86, and 0.89. Peak No. 10 in a methanolic extract of *Betula utilis* leaves covered the largest percentage area of 61.89% (R_f value, 0.86). Such an HPTLC method could be useful for the identification and evaluation of prepared materials, including leaves from *Betula utilis*. The reference standard was the marker chemical rutin.

The plates prepared at a wavelength of 365 nm were scanned using a CAMAG TLC scanner III in reflectance-absorption mode using a tungsten (W) light for both the sample and the reference. The data resolution was 100 µm per step, the slit size was 6.0 × 0.45 mm, and the scanning speed was 20 mm/s. The extract sample's HPTLC chromatograms using

the reference Rutin. the extract sample's HPTLC chromatograms using the reference Rutin. The plant sample's R_f and UV spectra were compared to marker chemicals with a 365 nm range in order to identify the marker compounds.

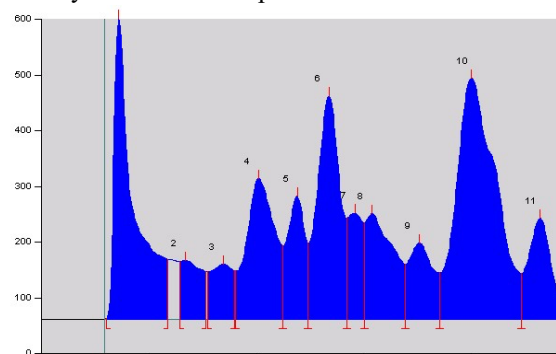


Figure 4 Methanolic extract of *Betula utilis* leaves displayed as an H.P.T.L.C chromatogram at 365 nm

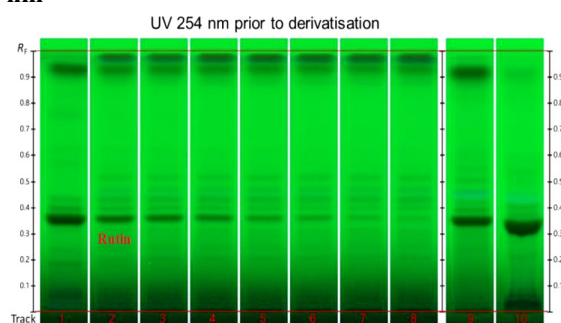


Figure 5 H.P.T.L.C chromatogram of the methanolic leaf extract from *Betula utilis*

2.6.2 Methanolic extract of *Annona squamosa* roots

The methanolic root extract of *Annona squamosa* had ten peaks in the 200–800 nm spectral region. This R_f value is found out as 0.19, 0.29, 0.36, 0.43, 0.43, 0.54, 0.63, 0.65, and 0.71. In a methanolic extract of *Annona squamosa* roots, the highest % area 61.95% was peaked at No.4 (R_f value, 0.43). Such an HPTLC method might be useful for the identification and evaluation of prepared leaves from *Annona squamosa* roots. Quercetin, a marker chemical, was employed as a benchmark.

In H.P.T.L.C chromatograms of extract sample with the standard Quercetin. The plant sample's R_f and UV spectra were compared to marker chemicals with a 365 nm range in order to identify the marker compounds.

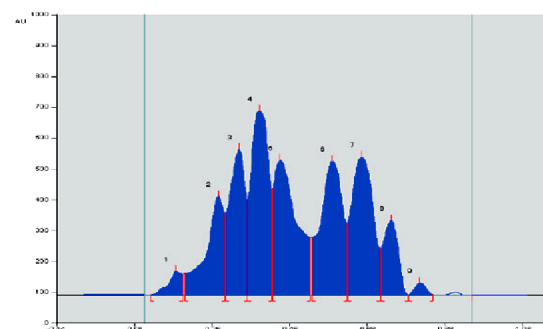


Figure 6 Methanolic extract of *Annona squamosa* roots displayed in an H.P.T.L.C chromatogram at a length of 365 nm

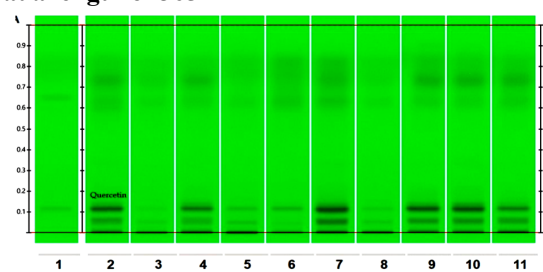


Figure 7 H.P.T.L.C chromatogram of *Annona squamosa* root methanolic extract

4. DISCUSSION

An exhaustive literature survey was conducted to identify gaps in current knowledge and to justify the selection of *Betula utilis* and *Annona squamosa*. Traditional texts have long mentioned these plants for their medicinal properties, including potential anticancer effects. Despite their documented uses, there is a lack of comprehensive studies examining their phytochemical and pharmacological profiles, particularly concerning colon cancer. This gap in the literature provided a strong rationale for our research. The authenticity of the plant samples was a critical step to ensure the reliability of the study. Plant specimens were collected from verified sources and authenticated using both botanical and molecular techniques. This step was crucial to eliminate any variability that might arise from using misidentified plant materials, thus ensuring the integrity of subsequent analyses. The establishment of preliminary pharmacogenetic standards for *Betula utilis* and *Annona squamosa* involved detailed macroscopic and microscopic examinations. These standards serve as a baseline for identifying and verifying plant materials in future studies. Alkaloids, flavonoids, saponins, and tannins were among the bioactive substances found during the phytochemical screening.

Future investigations should concentrate on identifying and characterising the precise bioactive substances causing these effects, as well as carrying out in-vivo tests to verify their safety and effectiveness. The development of standardized extracts and formulations could pave the way for new, plant-based anticancer drugs, offering hope for more effective and less toxic treatment options for cancer patients worldwide.

5. Conclusion

The preliminary pharmacogenetic evaluation established essential standards for the identification and verification of these plants, which is crucial for ensuring consistency in future research. Key bioactive substances, including phenolics and flavonoids, which are recognised for their antioxidant qualities, were found through phytochemical screening. Fractionation of methanolic extracts further highlighted the significant levels of these compounds in specific fractions. In conclusion, the pharmacogenetic and physiochemical evaluation of *Betula utilis* and *Annona squamosa* revealed some significant findings. The bioactive compounds responsible for anti-inflammatory, antimicrobial, antioxidant activities and anticancer were present in the phytochemical evaluation of these plants. The pharmacogenetic evaluation also revealed certain polymorphisms that may give the agent a potential effect on the impotence of this plant in efficacy and safety, respectively. This is conclusive proof that *Betula utilis* and *Annona squamosa* are a step forward in the development of new drugs and therapies. Nevertheless, more studies are necessary to completely understand pharmacogenetic and physiochemical properties of these plants.

6. Acknowledgements

Pragti Singh is a Ph.D. Scholar of Research at M.U.I.T, LKO. Authors are grateful to the M.U.I.T, CDRI (LKO), and BHU (Varanasi), for providing the facilities required to conduct this study.

7. Author Contribution

Research findings and experimental work are done by Pragti Singh, while work supervision and research findings drafting are done by Ajay Kumar Singh Rawat. Every author has read and approved the manuscript's published form.

8. **Conflict of interest:** The author(s) declare(s) that there is no conflict of interest.

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