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**UV-VIS SPECTROPHOTOMETRIC PROFILING OF SECONDARY METABOLITES
AND ISOLATION OF GENOMIC DNA OF ANTI-ALLERGIC CASTER OIL PLANT
(*RICINUS COMMUNIS* L.)**

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ABSTRACT

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Medicinal plants are essential to maintaining human health. There are over 7,500 species and 300 genera in the broad family Euphorbiaceae. They are typically the plants that bloom. The *Ricinus communis* plant, also known as the castor plant, is the most valuable in terms of traditional medicine and maintaining a healthy, disease-free existence. While the plant has multiple beneficial effects, including anti-oxidant, antihistamic, antinociceptive, antiasthmatic, antiulcer, immunomodulatory, antidiabetic, hepatoprotective, antifertility, anti-inflammatory, antimicrobial, central nervous system stimulant, lipolytic, wound healing, insecticidal, and larvicidal properties, it is traditionally used as a laxative, purgative, fertilizer, and fungicide, among other traditional uses. The plant exhibits this effect as a result of its significant phytochemical components, which include flavonoids, saponins, glycosides, alkaloids, and steroids, among others. This publication aims to provide future researchers with an explanation of the specific Phyto-pharmacological features of *Ricinus communis*.

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INTRODUCTION

It is a fact that human life is impossible without nature. Humans need three basic necessities: food, clothing, and shelter. One other need is good health, which is provided by the kingdom of plants. Organic chemicals found in plant kingdoms are abundant and have been utilised for medical purposes for a long time.¹ Natural crude drugs, such as *R. communis* L. (Family: Euphorbiaceae), also called "castor plant" or "palm of Christ," are used in traditional medicine to treat a wide range of illnesses and disorders. Other common names for this plant include Jada (Oriya), Verenda (Bengali), Endi (Hindi), Errandi (Marathi), and Diveli (Guajarati). As a

decorative plant, the plant is widely available in tropical climates.²

There are year-round, layered, natural, or agricultural trees up to 600 feet in height. The oil extracted from the seedlings' soil is stored safely. This is the best medication available for purifying cells. This is also the greatest anti-wind medication.³ In addition, it can be taken as medication to exacerbate the vata outbreak's constipation rate and, to a lesser extent, the vata problem.⁴ Our anguish eliminates it. It functions in conjunction with medication as part of nutrition. There are two varieties of castor blood.

Woody plants with larger seeds are burned, and those with smaller seeds are used for medicinal purposes. For decades, both

intentional and unintentional *R communis* L. poisonings in people and animals have been documented. However, the underlying cause of these poisonings remained unknown until 1888, when Stillmark linked the toxicity to the lectin ricin. *R communis* L.⁵ is industrially grown all over the world to produce castor oil. Ricine is mass manufactured in excess of a million tonnes annually as a by-product of the production of castor oil.⁶ A possible biological warfare agent, ricin has drawn interest due to its availability, toxicity, ease of manufacture, and the absence of medical countermeasures at this time. The seeds also contain the alkaloid ricinine, which is particularly useful for monitoring intoxications, and the less poisonous but highly similar *R communis* L. agglutinin.⁷

Following the process of oil extraction and detoxification, the defatted press cake finds application as low-value feed and organic fertiliser. In this regard, there have been occasional reports from various nations detailing animal intoxications following consumption of fertiliser that has been manifestly inadequately detoxified.⁸ However, over a number of years, observations in Germany have led us to hypothesise that the detoxification process is not always carried out completely and under strict control, necessitating international standards that explicitly indicate a ricin threshold in fertiliser.⁹ We provide an overview of the current understanding of both intentional and accidental ricin or castor seed poisoning in humans and animals in this study, with a focus on forensic analysis and intoxications brought on by inadequately detoxified castor bean meal.¹⁰

MATERIALS AND METHODS

Plant selection:

India and the Indian subcontinent are home to hundreds of different types of plants. It was difficult to choose a handful of these plants for this study's needs. If there is negligence in the plant selection, it will have a significant impact on the research project. Secondary metabolites in plants frequently collect in particular plant sections. Therefore, unless it is previously known which sections have the highest concentration of the relevant

chemicals, it is crucial to gather several plant sections or the entire plant in order to guarantee that the extracts produced are representative of the variety of secondary metabolites.¹¹ The following factors can be used to pick a sample for drug development from plants that will ensure the research project proceeds smoothly. It is clear from the literature review that there has been a great deal of research done on the plant *R communis* L. regarding chemical investigations of this plant, particularly with its leaves. I therefore had the opportunity to choose *R communis* L. leaves for my research project.

Plant collection

For scientific purposes, plant components must be collected after the plant has been chosen. The plant *R communis* L. is available across India. The leaves were gathered from Banat district Shamli's garden (U.P).

Drying and grinding of plant sample

To create the sample extract, the sample must be dried after collection. To prevent the breakdown of thermolabile chemicals, plant material should generally be dried at temperatures lower than 30°C. Sun drying is therefore highly beneficial, but there is a drawback: occasionally the sample absorbs water molecules, which can lead to microbial growth and interfere with the phytochemical analysis.

Cold Extraction with Shaking Method:

6 gm of powder is combined with 120 ml of methanol using a rotary shaker for 6-7 hours. After heating the crucible to 60°C in a hot air oven, we now weigh the empty crucible. We now grab filter paper and filter the crucible sample from the rotary shaker with the help of Whatman paper. The material was filtered into a crucible and then placed in a water bath to evaporate completely between 60-80°C. The extract that remains after evaporation is utilized in the experiment.

PRELIMINARY PHYTOCHEMICAL SCREENING:

The following phytochemicals (alkaloids, Tannins, Saponins.) were analyzed by using the Pharmacopoeia procedure in 1985.

THINLAYER CHROMATOGRAPHY (TLC):

Instrumentation and Experimental Procedures:

This plant contains flavonoids, saponins, alkaloids, carbohydrates, and tannins, among other compounds, according to a phytochemical investigation. To validate the presence of important groups such as alkaloids, flavonoids, saponins, etc. in the extract, this extract was subsequently submitted to TLC. Substances were separated according to their RF values¹². Dried extracts were redissolved in methanol after the solvent evaporated.¹³ The fraction was analyzed using TLC on a Merck Silica Gel 60 glass plate with several eluents. UV/VIS observations of the chromatograms were made both prior to and following the spraying agent processing.¹⁴ By comparing the flavonoids and phytochemicals to co-chromatographed standards and information from the literature, they were identified. 25 ml of chloroform and 25 ml of methanol were used to dissolve 42 g of silica gel.¹⁵ apply the gel over the TLC plates to prepare them. Pencil was used to mark the silica gel TLC plates. To "activate it," I put the TLC plate in the oven at 50–60°C for 15–20 minutes. Water molecules that attach to the polar locations on the plate are driven during activation. The slender capillary tip Touch the capillary on the silica plate that was dipped into the extract as soon as the extract rises into it cautiously.¹⁶ Permitted the solvent to evaporate entirely from the area. The TLC plate was very carefully inserted into the developing vial with the solvent system for the mobile phase. To allow the solvent front to move, I left it for a while. To evaporate the solvent, place the slide in an oven set to between 50-60°C. To obtain the banding profile of the *R communis* L. extract, two solvent solutions were used.¹⁷Water, Methanol, Butanol, and Chloroform (10:10:1:6).Light and sulfuric acid spraying (vanillin).The sample was placed on the plate and allowed to dry for a short while. After that, the solvent system was set up and given 10 minutes to stabilise.¹⁸ Following that, the plate was submerged up to three-fourths of the way in the solvent chamber. It was then taken

out and allowed to air dry. The plate was visually inspected.¹⁹

Microscopic Examination:

Microscopy of the leaf:

Trichomes, stomata, and epidermal cells are crucial leaf-identifying features. It is impossible to study their precise nature in the transverse section. Therefore, surface/epidermal exposure becomes crucial for the detailed microscopical analysis. The leaf fragment was removed by boiling it in chloral hydrate and removing the outermost layer of the epidermis. The epidermal piece was mounted in glycerin water and preserved on a slide. Numerous leaf characteristics were looked at. To make the design to scale, the drawing board and camera Lucida were arranged to extract the stomatal index and stomatal number. A cleared leaf was then placed on the slide after an inch of square was drawn using a stage micrometer. After tracing the epidermal cells and stomata, the number of stomata in a 1 sq. mm area was counted.

Microscopy of the stem:

Fresh stem microscopy was investigated. A transverse piece of the stem was obtained and stained with saffranin for microscopy. Sections were captured on photomicrographs. The sections that were manually cut and stained using various reagents were subjected to histochemical examination. The stem was first treated with chloral hydrate solution, then stained for 5-10 minutes in 1% saffranin, and finally mounted in 50% glycerine.

Qualitative phytochemical screening with UV-VIS spectrophotometer

Using a spectrophotometer, the Systronics UV-VIS Spectrophotometer 119, the qualitative analysis of various phytochemicals was performed. The analysis was based on the UV spectra obtained from the absorption maxima at each wavelength of the phytochemical in question. TT cytotype crude extracts were prepared using methanol of HPLC grade. Pure methanol was used for the calibration. A spectrophotometer was used to screen newly prepared samples after they were placed in a cuvette. The wavelength range of 195–550 nm was scanned for the absorption spectra of TT samples. Based on

previously published data, the absorbance of current phytochemicals was noted using the phytochemicals' λ -max.

DNA isolation studies on *R communis L.*

Plant material for DNA preparation

R. communis plant sample was utilized. The Banat district Shamli (U.P.) garden yielded the leaves of *R communis L.* The following information is taken from the appendix and describes the chemicals, buffers, and instruments used:

Reagents, chemicals and Solutions

1.5% (w/v) CTAB, 100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (pH 8), and 0.2% 2-mercaptoethanol were combined to create the extraction buffer. Furthermore, freshly prepared solutions of chloroform:isoamyl alcohol (24:1, v/v) and phenol: chloroform: isoamyl alcohol (25:24:1, v/v) were autoclaved for sufficient sterilization. Other solutions included RNase A (10 mg/ml), 3M sodium acetate, isopropanol, and 100 and 70% ethanol. Originally introduced by Doyle and Doyle in 1987, CTAB is widely utilized as a surfactant in DNA extraction processes. There have been some changes made to this experiment. The updated protocol provides the following:

Protocol for small scale preparation and purification of Genomic DNA :

After sterile distilled water was used to surface sterilize the leaves of *R communis L.*, 80% ethanol was added. In the presence of liquid nitrogen, dried leaf samples weighing 0.5 g were pulverized using a pre-chilled mortar and pestle. The frozen powder was put into a micro centrifuge tube along with 0.75 cc of the extraction buffer (pH 8). The frozen powder and extraction buffer were thoroughly combined, and the mixture was then incubated at 65°C for 30 minutes while being periodically shook in a water bath shaker. Following the incubation period, the mixture was allowed to cool to room temperature. An equal volume of the chloroform: isoamyl alcohol (24:1) combination was then added, and it was gently stirred for at least 15 to 30 minutes. After that, the mixture was centrifuged for 10 minutes at 10°C at 3000 rpm.

Equal volumes of ice-cold ethanol were added

to the aqueous phase to precipitate the DNA, and the mixture was then transferred to a clean, new microcentrifuge tube. For 30 minutes, the tube was kept at room temperature to allow the DNA to settle and precipitate. Centrifuging precipitated DNA for 10 minutes at 10°C at 5000 rpm. The supernatant was repeatedly pellet washed with 80% ethanol after being carefully decanted. After being dried in a laminar hood for 15 minutes at 37°C, the pellet was dissolved in 100 μ l of 1X TE using a dry pallet.

Purification of genomic DNA-

The main impurities in crude DNA production are polysaccharides, proteins, and RNA. The addition of CTAB to the DNA extraction buffer greatly aids in the removal of polysaccharides from DNA preparations. RNase is used to treat the samples in order to eliminate the RNA. Using proteinase to treat the sample will eliminate proteins, including RNase. In the DNA purification process, extraction with phenol: chloroform after RNase treatment is also used to remove RNA and the majority of the proteins listed below.

After treating the crude DNA sample with 10 μ l RNase (10 mg/ml stock), it was incubated for 30 minutes at 37°C in a water bath. The mixture of phenol, chloroform, and isoamyl alcohol (25:24:1) was then added in an equal volume. After that, it was centrifuged for 10 minutes at 25°C at 8000 rpm. Following the foregoing procedure, an equal amount of the chloroform:isoamyl alcohol (24:1) combination was added to the aqueous phase. This process was then repeated. After gathering the aqueous phase, it was combined with 1/10 volume of 3M sodium acetate and double volume of cold 100% ethanol to precipitate DNA. DNA Spooled out, twice-washed in 70% ethanol, and allowed to dry for 30 minutes at room temperature in a laminar flow environment.

In order to retain the DNA pellet for later use, it was finally dissolved in 50 μ l of 1x TE buffer and kept at -20°C. Ultimately, the DNA pellet was dissolved in 50 μ l of 1x TE buffer and kept for later use at -20°C.

Quantitation of genomic DNA

DNA quantitation is typically done by spectrophotometric measurements and agarose gel analysis. Reliable measurements of DNA concentration are important for many applications in molecular biology, including complete investigation of DNA by restriction enzymes and amplification of target DNA by polymerase chain reaction.

Spectrophotometric measurement-UV-VIS spectrophotometer (Systronics) was used to calculate the DNA yields per g of fresh leaf tissues. The ratio of absorbance at 260 nm to 280 nm was used to calculate the purity of the DNA.

- A cuvette containing one millilitre of TE buffer was used to calibrate the spectrophotometer at a wavelength of 260 nm.
- Added two to five microliters of DNA to a cuvette, mixed it well, and measured the optical density (OD) at 260 and 280 nanometers.
- The following formula was used to determine the amount of DNA in $\mu\text{g/mL}$:
DNA concentration ($\mu\text{g/mL}$) equals $(\text{OD})_{260 \text{ nm}} \times 50 \times \text{dilution coefficient}$.
- Analyse the DNA quality by comparing the OD values at 260 and 280 nm. A ratio of approximately 1.9 (1.8 to 2.0) suggests that the DNA that was isolated is of good quality.

Agarose Gel Analysis

By running the sample on an agarose gel stained with ethidium bromide solution ($0.5\mu\text{g/ml}$), the purity of the isolated DNA was verified.

- Pour agarose gel (0.4%) into a pH 8.0 (1XTBE, or Tris-borate-EDTA) buffer. Once the gel had hardened, move it to the electrophoresis tank containing 1XTBE tank buffer.
- After extracting 2–5 μl of DNA, combine it with 2 μl of bromophenol dye, and fill the wells.
- For 30 minutes, the electrophoresis was operated at 90V.
- Analyse the gel's DNA quality by looking for a single, compact band when exposed to UV light.

RESULTS AND DISCUSSION

The results of a variety of experiments were very good and showed that the phytochemical components of *R communis* L. are extremely broad. The phytochemical properties of *R communis* L. plant extracts were found to contain alkaloids, flavanoids, phenol, carbohydrates, tannin, phytosterol, saponins, and proteins, as confirmed by the test findings listed below.

Methanolic extract from rotary shaker method:

Initially, using a ROTARY SHAKER apparatus, 6 grammes of *R communis* L. L. root powder extract were combined with 120 millilitres of ethanol to create a methanol-based extract, which was then filtered using filter paper. After filtering and evaporating the transferred material in the crucible, the final weight was determined.

Ethanol : 120 , powder : 6gm

The dry, empty crucible weighs: 300.97 pounds.

Evaporated sample in a crucible: 301.56

Total : = 0.59 gm.

Table-1 : Methanolic extract from rotary shaker method

SR. NO.	NAME OF THE EXTRACT	NATURE	COLOUR	% YIELD(w/w)
1.	Methanolic extract	Shade	Green	7.38

First, the extracted work was made, and it was discovered that the rotary shaker approach produced a green, 7.38% methanolic extract.

Macroscopic Examination Leaves:**Table-2 : Macroscopic Examination-Leaves**

SR. NO	ORGANOLEPTIC CHARACTER	<i>R communis L.</i>
1.	Size	2.5 - 4 cm
2.	Surface characteristics, texture	Opposite arranged, ovate cordi form
3.	Taste	Little bitter
4.	Colour	Green
5.	Odour	Absent

R communis L. leaf macroscopy was performed; size, surface, features, texture, taste, and colour were not noted for examination or arrangement. It was discovered that *R communis L.* possesses an ovate cordi form with opposite arrangements. Liver is 2.5–4 cm in size, green in appearance, and mildly bitter.

Microscopic examination:

Equipment used = Optical microscope

1. A fresh leaf of *R communis L.* was examined under a microscope. There were wavy epidermal cells with anamocytic stomata, leaves with unicellular trachoma that

was simple to cover, lignified phloem, and pitted xylem arteries.

2. The stem of *R communis L.* was examined under a microscope. Because of the formation of interfascicular parenchyma between medullary bundles, successive cambium rings are found in patches rather than continuously. Microscopic analysis allows for a more thorough examination of the crude and makes it possible to identify the organised structural features, such as epidermis starch grains in endosperm and parenchymatous cells.

Preliminary phytochemical screening**Qualitative phytochemical analysis of *R communis L.*:****Table 3: Phytochemical constituents of *R communis L.* alkaloids and phenols are as follows:**

SR NO.	PHYTOCHEMICAL NAME	REAGENT TEST	OBSERVATION	TEST RESULT
A	Alkaloids	Mayer's reagent Wagner's reagent	Creamish precipitate / brownish red precipitate	+ +++
B	Phenols	2ml crude extract mixed with 2% soln. of fecl3	Yellow light coloration	++
C	Tannins	2ml crude extract mixed with 2% soln. of fecl3	Dark black coloration	+++
D	Lignins	Extract was treated with 2 % Furfurardehyde	Formation of red colour	++
E	Steroids	Crude extract mixed with 2 ml chloroform, then 2 ml of conc. H2SO4 shaken gently.	Reddish brown colour	++
F	Glycoside	Crude extract + 2ml of glacial acetic acid +1-2 drops of 2% soln of fecl3 poured another test tube contain 2ml of conc. H2SO4.	Formation of brown ring at the interphase.	+++
G	Saponins	Crude extract treated with 5 ml of distilled	Foam produced	+++

		water, shaken vigorously.	
H	Terpenoids	Aqueous extract + 2ml chloroform + 1ml conc. H ₂ SO ₄	NO COLOUR

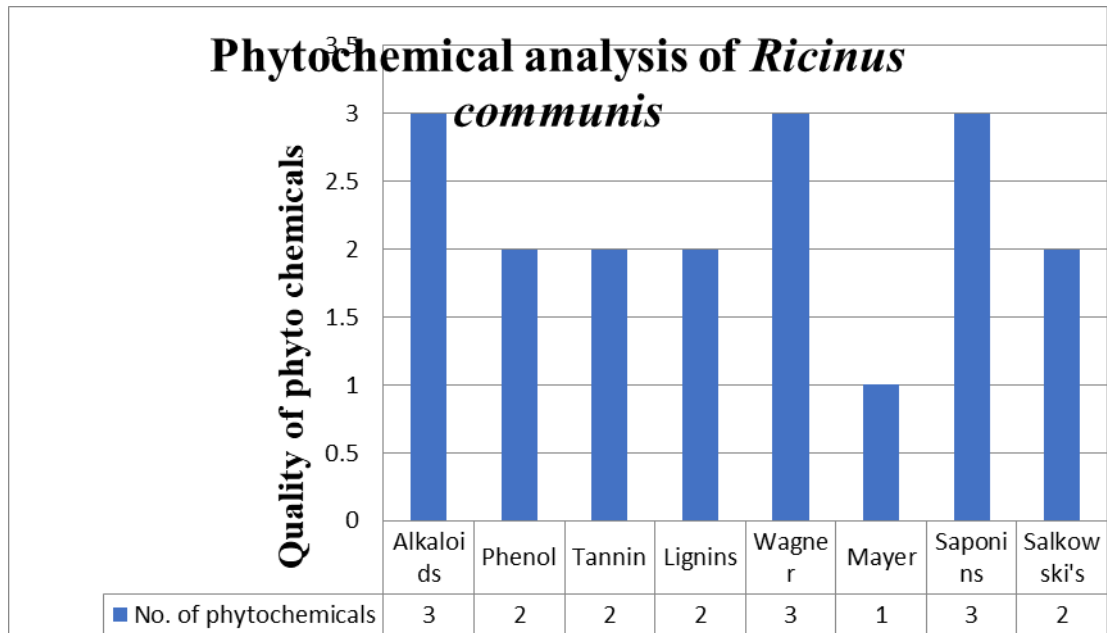


Fig 1: Phytochemical estimation of *R communis L.*

TLC (THIN LAYER CHROMATOGRAPHY)

Chromatographic studies:

Table-4: TLC with solvent system I Chloroform: Methanol (7.5:0.5)

SR. NO	PLANT SPECIES	EXTRACT	DISTANCE TRAVELLED BY SOLUTE (cm)	DISTANCE TRAVELLED BY SOLVENT (cm)	COLOUR	Rf value
1.	<i>R communis L.</i>	Methanolic extract	1 cm	6	Amber green	0.16
			1.3 cm	6	Spice green	0.21

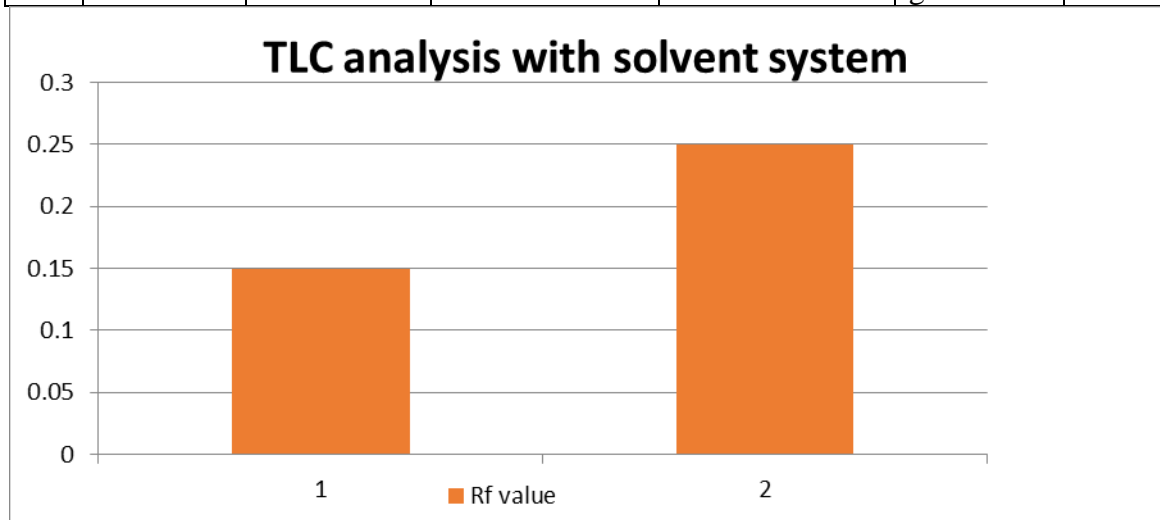


Fig 2 : Analysis with solvent system I Chloroform: Methanol (7.5:0.5)

Following the visualization process in TLC analysis using solvent system 1, two spots with respective R_f values of 0.16 and 0.21 were identified. Following the visualization procedure, five spots were seen in the second solvent system, with corresponding R_f values of 0.23, 0.33, 0.68, 0.71, and 0.75. A microscopic analysis of the stem and leaves was conducted. The outcome of both macroscopic and microscopic analysis could be helpful in differentiating it from adulterants and substitutes. The qualitative and quantitative phytochemical screening of the

plant extract often provides the necessary information regarding the chemical ingredient for the pharmacological discovery. Saponins, alkaloids, glycosides, and flavonoids were among the phytochemicals found in the extract. Only by analyzing the R_f values of compounds in the various solvent systems in the current state TLC profiling of all the plant extract of the leaves in the various solvent systems indicating the presence of diverse range of phytochemicals in the plant can the right solvent system be selected for a given plant extract.

Table- 5: Screening of phytochemical with UV-VS Spectrophotometry

WAVELENGTH	ABSORBANCE
320	0.687
340	0.708
360	0.843
380	0.509
400	0.317
420	0.202
440	0.075

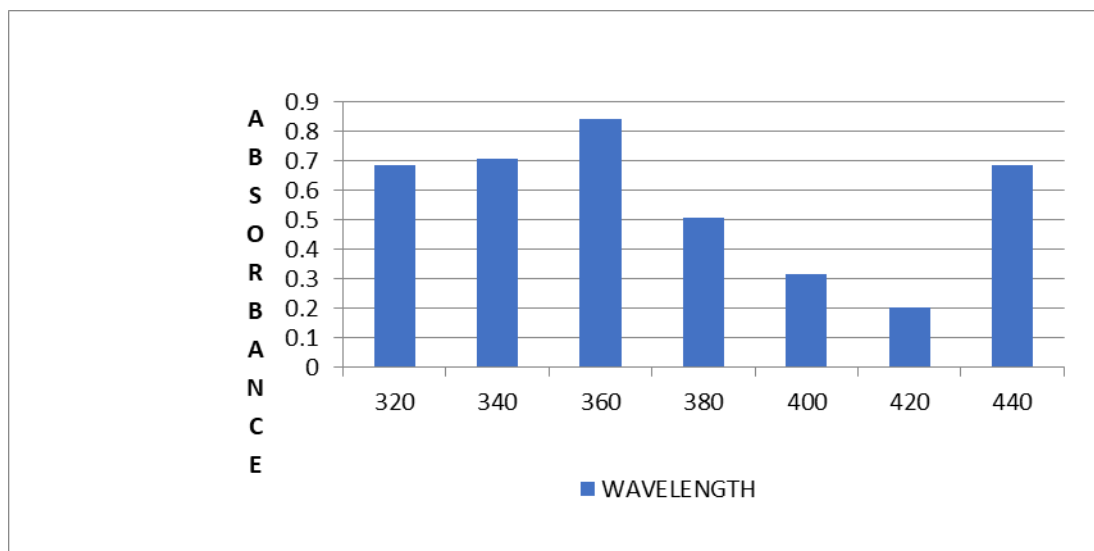


Fig 3- Screening of phytochemical with UV-VS Spectrophotometry

DNA isolation

It is preferable to use molecular markers for genotypic variation analysis when screening accessions, selecting parents, and selecting progeny. Certain plant compounds can interfere with DNA isolation processes and reactions, including cloning and PCR amplification. In order to overcome frequent issues including low yield, degradation, and poor PCR amplification, we employed the

CTAB technique to isolate DNA from leaves of the flavonoid-rich *R communis* L. We were able to analyse *R communis* L. DNA successfully. A crucial part of our process involved completely disrupting the plant cells in liquid nitrogen. High amounts of flavonoids and polyphenolics were precipitated using high concentrations of phenol and NaCl. *R communis* L. tissues yielded DNA at a rate of about 80 ng per gramme of tissue. According

to Pich and Schubert (1993), there were no pollutants present in the A260/280 ratio. Following the addition of alcohol during DNA separation, flavonoids and polyphenolics also co-precipitate with DNA, resulting in viscous solutions (Do and Adams, 1991), which renders the DNA unsuitable for restriction and Southern hybridization. PCR amplification of the extracted DNA was used to verify the

authenticity of the sample. A good amplification of genomic DNA was the outcome. This is a quick, easy, and effective way to separate DNA from plants that contain a lot of phenolic chemicals. It can be done at room temperature and doesn't require laborious, complex ultracentrifugation. Agarose gel electrophoresis was used to verify the isolation and amplification of DNA.

Table- 6: Physiochemical analysis of *R communis L.* crude drug

SR. NO	PLANT EXTRACT	REAGENT TEST	OBSERVATION
1.	<i>R COMMUNIS L.</i>	Powder+5% ammonia sol ¹	LIGHT BROWNISH
2.		Powder + 5% Picric acid	YELLOWISH
3.		Powder + 5% Ferric acid	YELLOW BROWNISH
4.		Powder + 5% H ₂ SO ₄	LIGHT BRICK RED
5.		Powder + 5% NaOH	LIGHT BROWNISH
6.		Powder + 5% Glacial acetic acid	LIGHT BROWNISH
7.		Powder + 5% KOH	LIGHT BROWNISH
8.		Powder + 5% HNO ₃	LIGHT BROWNISH
9.		Powder + 5% conc. HCl	BROWNISH BLACK
10.		Powder as such	BROWN TO YELLOWISH

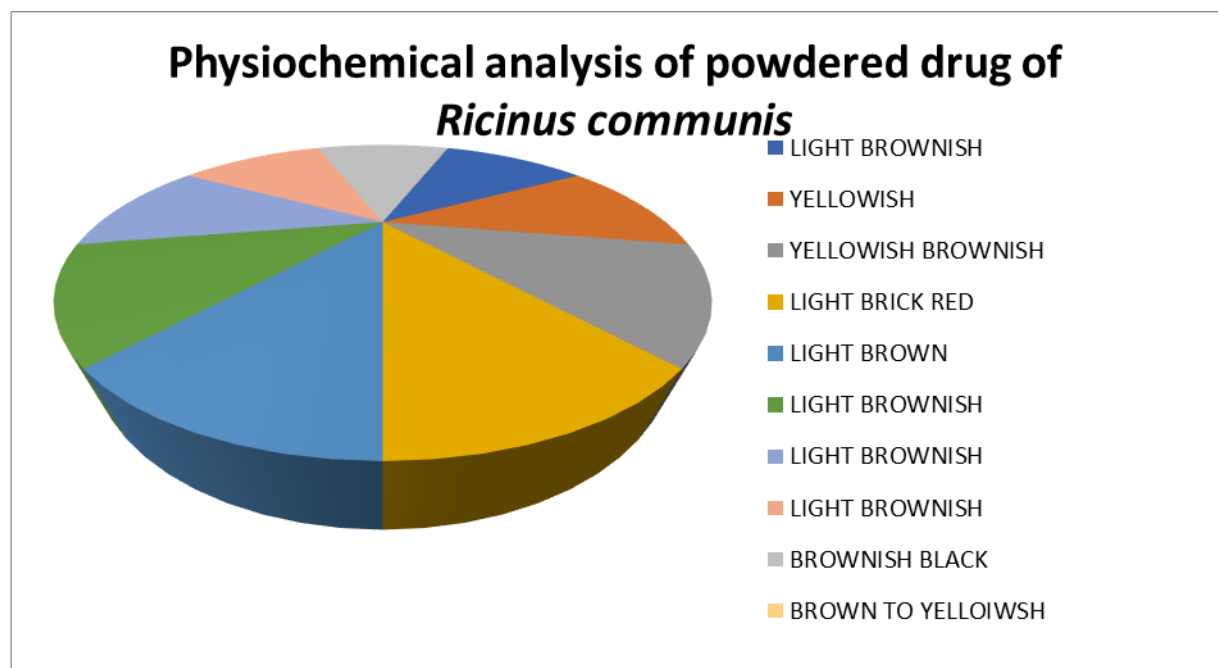


Fig 4 - Observation of different colours for Physiochemical analysis of powdered drug of *R communis L.*

When treated with various reagents, the powdered medication of *R communis L.* undergoes a physicochemical test for the presence of secondary metabolites and colour observation. The results are as follows.

CONCLUSION

Hemp, or *R communis L.*, is a type of plant in the Euphorbiaceae family that is used worldwide to treat a variety of alignment-related conditions. Patients who received anti-allergic medication were more likely to see a noticeable improvement in their pain levels. Oral cannabis were linked to an improvement in the reported symptom of muscle spasms in persons with multiple sclerosis. Oral cannabis are effective in treating and avoiding nausea and vomiting brought on by chemotherapy. After conducting a qualitative screening for phenol, tannin, oil and fat, and saponins, the following trend was observed, pointing to a pattern: phenol=tannin=oil & fats>carbohydrate>saponins. The amount of DNA extracted from *R communis L.* tissues was roughly 1.7 ng per gramme. In Following the visualization process, the solvent systems for sport in the TLC analysis were observed, with values of 0.1, 0.3, 0.5, and 0.9, respectively. We examined relevant data that indicated the existence of numerous phytochemicals at various wavelengths in the plant extract's US-VIS spectrum. At wavelengths of 320, 340, and 360 nm, absorbance values of 0.687, 0.708, and 0.843 indicate the presence of saponins and alkaloids, respectively.

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