



Phytochemical Analysis and Cardioprotective Activity of *Ricinus Communis* Leaf Extract Against Oxidative Stressed Induced Damage

Ajay Kumar singh^{1*}, Raj K. Prasad¹, Manoj K. Mishra¹, Anurag singh¹, Chandra P. Sharma¹, Shubham Yadav¹

¹Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj

Corresponding Author

*Ajay kumar singh, Research Scholar, Shambhunath Institute of Pharmacy, Jhalwa, Prayagraj, U.P., 211002

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KEYWORDS

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oxidative stress

ABSTRACT:

Ricinus communis is a traditional herbal remedy that has been used for ages to treat a wide range of illnesses. Summarizing *Ricinus communis* therapeutic qualities, potential use as a herbal remedy, and pharmacovigilance evaluation are the objective of this study examines the potential cardioprotective effects of extract from the leaves of *Ricinus communis*, or castor beans. The study investigates this plant's potential to fight oxidative stress, which is a major contributor to the emergence of cardiovascular illnesses. Using solvents such as ethanol, hexane, and distilled water, *Ricinus communis*, which is recognized for its diverse medicinal characteristics, was submitted to phytochemical analysis and extraction procedures. DPPH radical scavenging activity was one of the techniques used to assess the extracts' antioxidant potential. In order to evaluate the cardioprotective effects, the study used an experimental design with Wistar rats and metrics such as oxidative stress markers and haematological analysis. The findings suggested that the *Ricinus communis* leaf extract may be useful in preventing or lessening cardiovascular damage because of its strong antioxidant and cardioprotective qualities. The plant's therapeutic qualities are attributed to a variety of phytochemical components, such as glucosides, alkaloids, steroids, saponins, and flavonoids. study highlights include antioxidant activity, anti-inflammatory, and anti-diabetic effects of the plant. It also talks about how the plant may be used to treat illnesses like liver damage, and insecticidal properties. *Ricinus communis* is a highly advantageous medicinal plant that has been used for millennia to cure a variety of disorders, according to the study overall conclusion. It may be explored further as a potential herbal remedy in the future.

1. INTRODUCTION

Cardiovascular disease

The term "cardiovascular disease," or "CVD," is well-known to most people, yet it primarily describes illnesses that impact the heart and its parts, including myocardial infarction, angina pectoris, hypertension, stroke, and other circulatory problems. Reports have identified common heart disorders such as peripheral artery disease, arrhythmias, cardiac arrest, congestive heart failure, and coronary artery disease (Koshma et al., 2018)

Anticancer medications' cardiotoxic side effects have caused heart failure and myocardial dysfunction in a number of people. Cardiotoxicity has recently been associated with an imbalance in redox signaling. (Cadeddu Dessalvi et al., 2021)

IMPORTANCE OF CARDIOVASCULAR HEALTH

Frequent exercise has many known advantages, one of which is that it helps to maintain cardiovascular health. In order to inform exercise recommendations and support future research, it is critical to provide basic definitions and descriptions of physical activity and physical fitness.



Systematic reviews demonstrated the clear positive impact of physical activity on mortality from all sources. The pooled risk reductions comparing high and low levels of physical activity were 35% for cardiovascular mortality and 33% for all-cause mortality, even after adjusting for other relevant risk variables (Vanhees et al., 2012)

RISK FACTOR OF CARDIOVASCULAR DISEASE

- high blood pressure,
- high blood cholesterol,
- tobacco use (smoking and chewing),
- diabetes mellitus, and obesity.
- Low consumption of fruits and vegetables

2. Plant profile

The tropical plant *Ricinus communis*, commonly referred to as castor bean, is found all over the world. The plant is indigenous to India, where it is grown in fields and gardens as well as in untamed areas and waste locations. *Ricinus communis* is a little timber tree that is native to South Africa, India, Brazil, and Russia. It reaches a height of roughly six meters. *Ricinus communis* stems possess antitubercular, antidiabetic, and antiprotozoal properties. The plant's leaves, roots, and seed oil are used in Indian medicine to treat liver problems, inflammation, hypoglycemia, and laxative effects.

The leaves are deciduous, with a blade that is 6–8 inches across and is palmately cut for three quarters of its depth into 7–11 lanceolate, acute, coarsely serrate segments. The leaves are alternate, curved, cylindrical, purplish petioles, sub-peltate, drooping, and with large, ovate, yellowish stipules that unite into a cap enclosing the buds. The leaves are smooth blue-green, paler underneath, red, and shining when they are young. The monoecious nature of flowers (Maciel-Torres et al., 2024)

3. MATERIAL AND METHOD

3.1 Identification collection and authentication of plant material

The plant material (leaf) of *Ricinus communis* was collected from the suburbs of (teliarganj) prayagraj, uttar Pradesh, India, in the month of March 2024 and the plant was identified and Authenticated by Botanical Survey of India, Prayagraj, uttar Pradesh. The specimen of *Ricinus communis* leaf was certified by Mr Vijay Ranjan, Scientist-E head of office. Botanical survey of India Central Region; I centre, Chatham lines, Prayagraj 211002. A specimen at the Botanical Survey of India, Prayagraj, India.

3.2 Pharmacognostic study of plant.

Table no.1 Characterization for Leaf of *Ricinus communis*

s.no	Organoleptic charactor	<i>Ricinus communis</i>
1.	Size	7.10–32.57 x7.89–34.39 cm
2.	Surface, characteristics, texture	glabrous, more or less flat, and elliptic
3.	Taste	Bitter
4.	Colour	Green
5.	odour	characteristic

3.2.1 Microscopic feature of leaf

The elliptic, alternating, green, glabrous, and somewhat flat leaves measured $7.10-32.57 \pm 7.89-34.39$ cm (Table 2). The leaf area was between 225.14 and 5000.19 mm², and the leaf blade classes were classified as macrophyll (<1820 mm²) and mesophyll (225–1820 mm²) in Table 2. *Ricinus communis* leaves can have seven to thirteen lobes per bush, and they can be symmetric or asymmetrical. The leaves have a palmatipartite incision and are simple, lobed leaves. The leaf's incision percentage, with a curved sinus, ranges from 52.28 to 80.64% (in samples 1 and 21, respectively). 1.49–7.83 cm was the size of the first lobe (central lobe),



4.60–21.49 cm was the size of the second pair, and 3.86–17.84 cm was the size of the third pair (Nour et al., 2023)

3.3 Extraction

3.3.1 Soxhlet extraction

Ethanol, hexane, petroleum ether, and distilled water were used in the Soxhlet extractions. The solvents selected for this investigation are often used to extract oil from the leaves of *Ricinus communis*. A round-bottom flask was filled with 100 mL of solvent, and 25 g of leaf was added.

The heating mantle was positioned inside the flask. The condenser was attached to the extractor after the extractor was linked to the flask. Following the boiling and evaporation of the solvent, the vapor was condensed and poured into the extractor. The solvent seeped back into the flask with a circular bottom. For four to six hours, this evaporation-condensation process went on. After that, the device was let cool. To eliminate the solvent, the cold solvent-seed oil mixture was poured into a beaker and baked at 700 degrees Celsius. Oil from *Ricinus communis* seeds was extracted using the Soxhlet method.



Fig 1. Soxhlet apparatus

3.4 Determination of physicochemical property

3.4.1 Loss on drying

Weight reduction is expressed as a percentage of body weight. It computes the volume of any volatile substance that can be driven off under the specified circumstances (desiccator or hot air oven), including water. If the sample is in the form of large crystals, reduce its size by quickly crushing it into a powder..

$$\text{Loss of drying} = \frac{\text{initial weight of sample} - \text{weight of sample after drying}}{\text{initial weight}} \times 100$$

3.4.2 Swelling index

The swelling index is defined as the volume measured in milliliters (ml) that results from treating a specific quantity of air-dried plant material (1 gram) with an appropriate solvent (25 ml). 1 gram of air-dried plant material is taken and transferred into a 25-ml granulated stopper measuring cylinder, with each division holding 2 ml. The length of the 1 gram of air-dried material in the measuring cylinder, which has a length of 125 mm and an internally examined the plant material's length that appears in the solvent's top and lower middle portions to ascertain diameter of 60 mm, is then measured. 25 ml of water is added, and it is shaken occasionally for at least one hour before being left to stand for the following three hours. Carefully examined the plant material's length that appears in the solvent's top and lower middle portions to ascertain

3.4.3 Ash value:

3.4.3.1 Determination of total ash

Weighing out around 2 grams of the medication, we put it in a china dish and put it in the incinerator, where it burned for 5 to 10 minutes at 450 °C. After cooling and weighing the residual ash, the proportion of ash is determined using dry medication (Tamboli et al., n.d.-a)

$$\% \text{ total ash value} = \frac{\text{weight of ash}}{\text{weight of drug}} \times 100$$



3.4.4.1 Acid insoluble ash

The remaining ash in the whole ash is filtered using 25 milliliters of dil HCL, leaving behind acid-insoluble ash residue on filter paper. The percentage is then computed using the dried crude drug.

$$\begin{aligned} &\% \text{ Acid – insoluble ash value} \\ &= \frac{\text{Acid insoluble ash weight}}{\text{weight of drug}} \times 100 \end{aligned}$$

3.4.5.1 Water soluble ash

After dissolving the entire amount of ash in 25 milliliters of distilled water and filtering the ash solution, the water-soluble ash is calculated as a percentage of the total amount of ash and deducted from it. This results in dry medication.

Identifying the extractive that is alcohol-soluble Weigh five grams of the drug, place it in contact with one hundred milliliters of alcohol, and let it macerate for twenty-four hours while shaking it occasionally. After that, filter the filtrate, evaporate it until it is completely dry, and use the dried drug to compute the percentage of alcohol-soluble extractive(Tamboli et al., n.d.-a)

$$\begin{aligned} &\% \text{ of water soluble ash} \\ &= \frac{\text{Dish residue weight} - \text{Dish weight}}{\text{weight of sample}} \times 100 \end{aligned}$$

3.4.5 Identification of extractive value

3.4.5.1 Alcohol soluble extractive value

Accurately weighted 5 grams of air-dried crude drug was taken in a closed flask and macerated with 100 ml of 95 percent ethanol for 24 hours. Shaken frequently during the first 6 hours and filter rapidly, taking precaution against loss of alcohol. 25 ml of the filtrate was taken,

them, and their cages were always maintained odor-free and clean to prevent needless stress(Adeoye et al., 2023)

Research has been conducted on the therapeutic and toxicological characteristics of leaf extracts from *Ricinus communis*. Leaves of *Ricinus communis* contain a variety of bioactive substances that are thought to be necessary

evaporated to dryness in a water bath, and dried at 105 °C. Then cool in the desiccator and weigh. The percentage of alcohol-soluble extractive value was calculated with reference to an air-dried drug(Shaikh, 2022)

$$\begin{aligned} &\text{Alcohol soluble extractive value \%} \\ &= \frac{\text{weight of extract}}{\text{weight of drug}} \times 100 \end{aligned}$$

3.4.5.2 Water soluble extractive value

For 24 hours, 5 grams of the coarsely ground, air-dried medication must be macerated in a closed flask with 100 milliliters of water, stirring frequently for the first 6 hours and letting stand for the remaining 18 hours. After that, filter quickly while being cautious not to lose any water, evaporate 25 milliliters of the filtrate in a shallow dish with a tarred bottom, dry at 1050 C, and weigh. Calculations are made to determine the percentage of water-soluble extractive value in relation to the air-dried drug stop(Shaikh, 2022)

$$\begin{aligned} &\% \text{ Water soluble extractive value} \\ &= \frac{\text{weight of extract}}{\text{weight of drug}} \times 100 \end{aligned}$$

3.5 ACUTE TOXICITY STUDIES

In this investigation, thirty (30) male Wistar rats were employed. M/S Chakraborty Enterprise in Kolkata provided the animals (registration number 1443/PO/Bt/s/11/CPCSEA). The creatures weighed 200–250 grams. They were kept in plastic cages at the Animal House for two weeks while they were acclimated to the proper environmental circumstances, which included regular cycles of twelve hours of light and darkness. Standard grower's mash rat pellets were given to

for any medicinal plant, including terpenoids, tannins, flavonoids, cardiac glycosides, steroids, saponins, and phlorotannis. When tested on rats at doses of 100 and 200 mg/kg body weight, a methanolic extract of *Ricinus communis* leaves was shown to be non-toxic and to have no adverse effects on critical organs. We can therefore conclude that, when ingested in moderation, the plant's



leaves are both safe to eat and possess antibacterial characteristics. When swallowed or inhaled, seeds are extremely poisonous, in contrast to leaves and other plant parts (Chouhan et al., 2021)

3.5.1 Isoprenaline induced cardiac damage in rats

Rats were injected subcutaneously with 100 mg/kg of isoprenaline hydrochloride dissolved in saline once a day for two days in a row to induce MI. The dose and route that were chosen were taken from published literature

3.5.2 Experimental design for cardioprotective activity

The rats were separated into five groups, with six individuals in each group, as follows:

Group 1: Normal control rats given only water.

Group 2: negative control (only isoprenaline) 2 days

Group 3: 7 days standard (ramipril) drug then isoprenaline (2 days)

Group 4: test 1 (low dose) 7 days, plus isoprenaline

Group 5: test 2 (high dose) 7 days, plus isoprenaline

3.5.3 DPPH Radical scavenging activity:

a) Preparation of DPPH reagent

0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

b) Preparation of Sample/Standard

Freshly 1 mg/ml methanol solution of all extracts/standard was prepared. 1 mg of extracts/standard was taken with methanol to make 1mg/ml stock solution. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

C) Preparation of control

For control, Take 3 ml of 0.1mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm (Athavale et al., 2012). Percentage antioxidant activity of sample/standard was calculated by using formula:

$$\% \text{ Inhibition} = \frac{\text{Ab of control} - \text{Ab of sample}}{\text{Ab of control}} \times 100$$

3.6 HPTLC study

For the qualitative, semi-quantitative, and quantitative phytochemical examination of herbal medications and formulations, HPTLC has become a crucial technique. This includes estimating biomarkers and creating TLC fingerprint profiles. This review aims to concentrate on the theoretical aspects of HPTLC and provides instances of herbal medications and formulations that have been subjected to HPTLC analysis (Tamboli et al., n.d.-b)

3.7 FTIR study

After the material came into contact with a sodium chloride screen, 32 images were combined to get FT-IR spectra with a frequency of 4500–400 cm⁻¹ and a resolution of 4 cm⁻¹. We limited each spectrum by comparing it to a background spectrum. A fresh reference background spectrum was found in every scan. At each data point, these spectra were triple-recorded as absorbance values (Mustapha et al., 2021)

One spectroscopic technique that has been combined with HPTLC for the identification and characterization of unknown samples is infrared (IR). The primary advantages of infrared spectroscopy are its rapidity and lack of reagent requirements, as well as the minimal to no sample preparation required before use. (Vyas et al., 2023)



4. RESULT:

4.1 Standardization:

Table no.2 Characterization for Leaves of *senna alata*

s.no	Organoleptic character	<i>Ricinus communis</i>
1.	Size	7.10–32.57 x7.89–34.39 cm
2.	Surface, characteristics, texture	glabrous, more or less flat, and elliptic
3.	Taste	Bitter
4.	Colour	Green
5.	odour	characteristic

4.2 Microscopic examination:



Fig 2. Compound Microscopic of *Ricinus communis*

4.3 Extractive Value of *Ricinus communis*

Table 3: Percentage yields in different solvent

Solvent	% yield
N-hexane	8.1
Ethyl acetate	19.2

Table 4: Physiochemical parameter analysis of Ethyl acetate's extract

S.N.	parameter	Observed value %
1.	Total ash value	10.5
2	Acid insoluble ash value	0.65
3	Water soluble ash value	0.1
4	Loss of drying at 105 °C	8.33
5	Water soluble extractive value	0.1
6	Alcohol soluble extractive value	5
7	Swelling index	0.021

4.4 DPPH activity

Table no.5 DPPH radical scavenging activity of extract

Concentration	Absorbance	% Inhibition	IC 50
20	0.625	32.867	
40	0.598	35.767	
60	0.574	38.345	
80	0.561	39.742	
100	0.543	41.675	
control	0.931		
IC50	175.7		27.93µg/ml

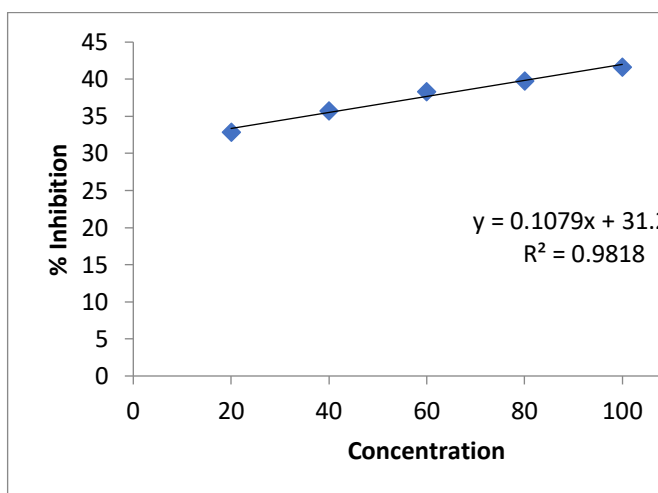


Fig 3. Graph 1 Graph represents the Percentage Inhibition Vs Concentration of extract



Fig 4. DPPH test

Physicochemical parameter analysis:

Table N0.6 Table shows the physicochemical investigation of *Ricinus communis* leaf

S.no.	Parameter	%w/w
1.	Total ash value	10.5
2..	Acid insoluble ash value	0.65

Pharmacological study model

3.	Water soluble ash value	0.1
4.	Loss of drying at 105 degree celcius	8.33
5.	Swelling index	0.021

Table no.7 Percentage yield (%)

s.no	Extractive yield	%wt/wt
1	Alcohol soluble extractive value	6.80%
2	Water soluble extractive value	17.80%

Table No.8 Percentage yield in different solvent

s.no.	solvent	Percentage yield (%)
1.	N-hexane	8.1
2.	Ethyl acetate	19.2
3.	Methanol	18.3

Chromatographic studies:

Table no.9 Inference of qualitative chemical examination of extract:

S.no.	Phytochemical constituent	Observation	Ethanol extract
1.	Alkaloid	Cream colour ppt	+
2.	Saponin	Dirty green ppt	+
3.	Flavanoids	Pale brown ppt	+
4.	Steroid	Red colour	+
5.	Resin	Reddish brown ppt	+
6.	Tannin	Dirty green ppt	+
7.	Protien	Red ppt	-
8.	Carbohydrate	Orange red ppt	-

Table no.9

**Table no 10:** haematological parameter Analysis

Parameters	Unit	Mean \pm SEM				Reference range
		Control group	Dose 1 (75 mg/kg)	Dose 2 (150 mg/kg)	Dose 3 (350 mg/kg)	
Haemoglobin	gm/dL	11.16 \pm 1.73	12.4 \pm 1.11	12 \pm 1.4	13.16 \pm 0.35	11-15
Total Leucocyte Count (TLC)	Cells/mm ³	4971.66 \pm 1766.19	4266.66 \pm 1464.01	5066.667 \pm 929.1573	5016.67 \pm 275.37	4000-10000
Neutrophils	%	23.66 \pm 8.60	16 \pm 1	17.66 \pm 1.52	16 \pm 2	20-45
Lymphocytes	%	76 \pm 4.21	72.66 \pm 8.62	45.33 \pm 8.32	52.33 \pm 4.72	25-40
Monocytes	%	4.66 \pm 2.51	2.33 \pm 1.25	3.06 \pm 0.45	3.6 \pm 0.45	2.0-10
Eosinophils	%	3.66 \pm 1.52	1.7 \pm 0.75	2.46 \pm 0.60	2.66 \pm 0.05	1.0-6.0
Basophils	%	0.03 \pm 0.05	0 \pm 0	0 \pm 0	0 \pm 0	Up to 0.6
Absolute Neutrophils Count (ANC)	10 ³ /ul	1.96 \pm 1.47	0.83 \pm 0.40	1 \pm 0.17	1.09 \pm 0.11	2-7
Absolute Lymphocytes Count (ALC)	10 ³ /ul	2.56 \pm 0.15	2.4 \pm 0.25	2.63 \pm 0.64	2.63 \pm 0.15	
Absolute Monocytes Count (AMC)	10 ³ /ul	0.43 \pm 0.30	0.166 \pm 0.15	0.13 \pm 0.05	0.2 \pm 0.1	0.2-1.0
Absolute Eosinophils Count (AEC)	10 ³ /ul	0.13 \pm 0.05	0.03 \pm 0.05	0 \pm 0	0 \pm 0	
Platelet Counts	Lac cells/mm ³	503666.67 \pm 57726.366	589333.3 \pm 249458.1	711000 \pm 42508.82	589667 \pm 55734.5	1,50,000-4,50,000
RBC Count	Million cells/ μ L	4.93 \pm 0.15	2.56 \pm 1.41	3.96 \pm 0.40	3.16 \pm 0.30	4.0-6.0
Packed Cell Volume	%	24.53 \pm 2.80	24.33 \pm 7.02	35.33 \pm 3.51	42.33 \pm 2.51	38-54
M.C.V.	femtolitres	81.13 \pm 2.0	74.02 \pm 9.90	79.66 \pm 10.88	84.06 \pm 5.64	76-96
M.C.H.	pico-grams	28.16 \pm 1.24	28.16 \pm 2.75	30.83 \pm 4.64	18.25 \pm 15.68	23-32
M.C.H.C.	g/dL	34.6 \pm 2.2	32.66 \pm 2.75	32 \pm 3.60	27 \pm 1	30-35



RBC Distribution Width -CV (RDW-CV)	%	14 ± 1	14.66 ± 1.52	13.66 ± 1.52	15 ± 2.64	
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HPTLC study

HPTLC result was found like rf value, area, and calibration curve reported in fig no. and table no.

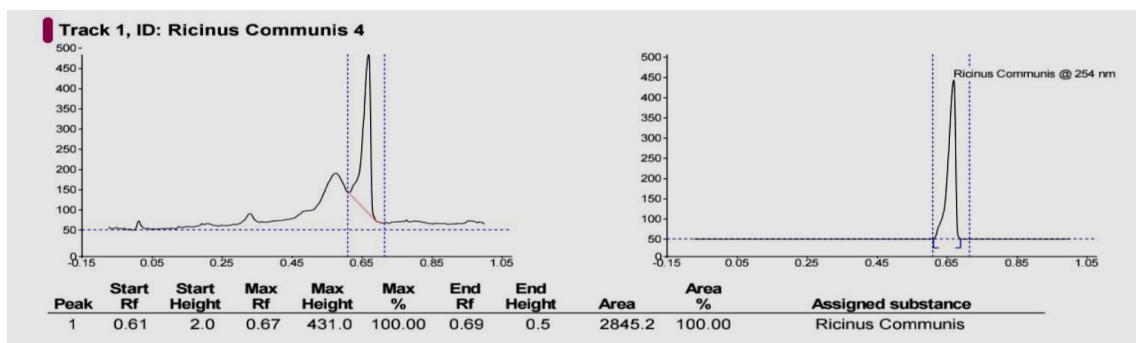


Fig no. 5

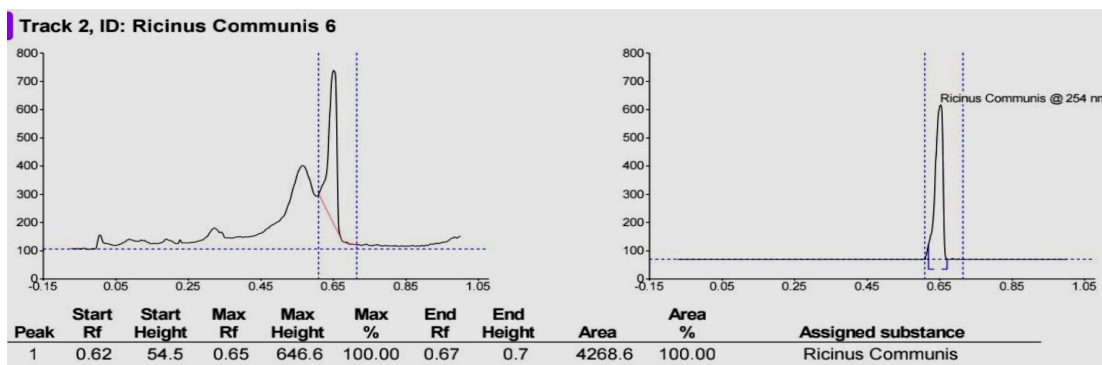


Fig no. 6

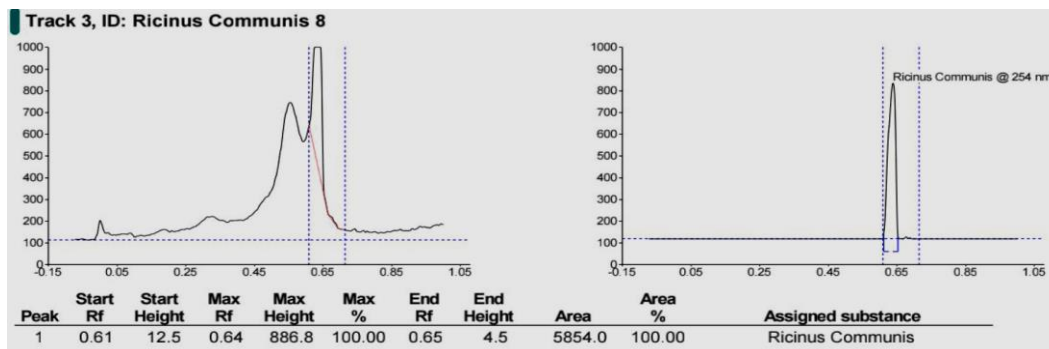


Fig no. 7

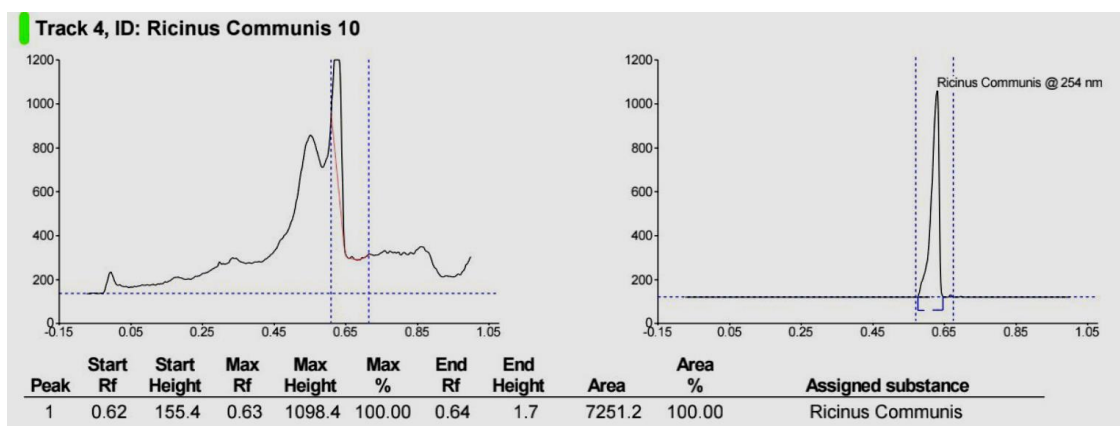


Fig no. 8

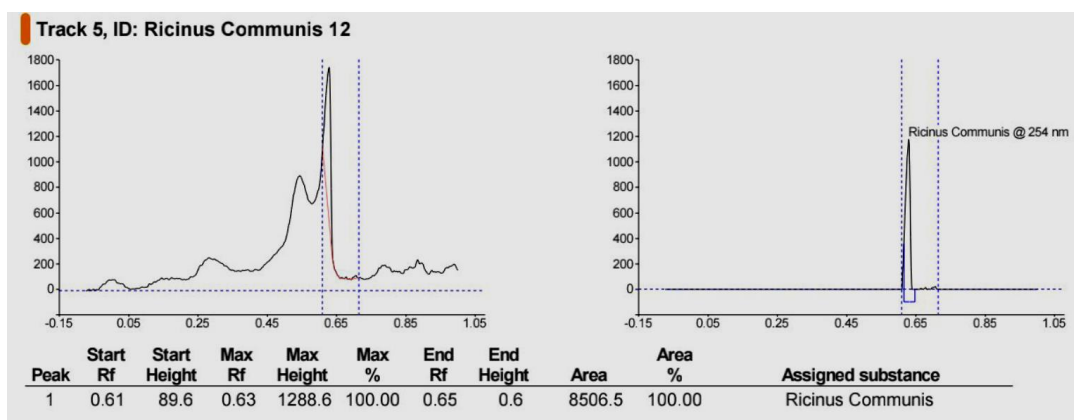


Fig no.9 HPTLC Spectra of *Ricinus communis* extract (4-12 µg/ml)

S.no	Concentration µg/ml	Area
1	4	2845.21
2	6	4268.58
3	8	5854.02
4	10	7251.19
5	12	8506.48

FTIR study:

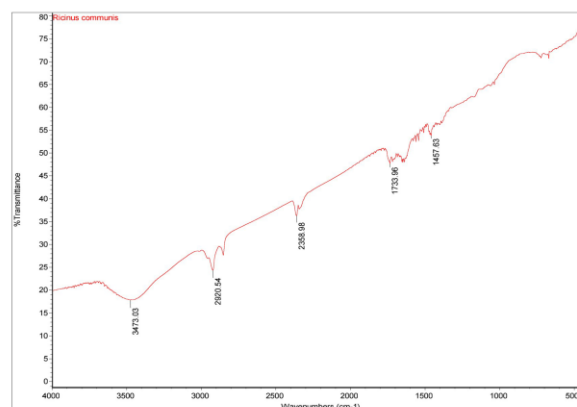


Figure no.13

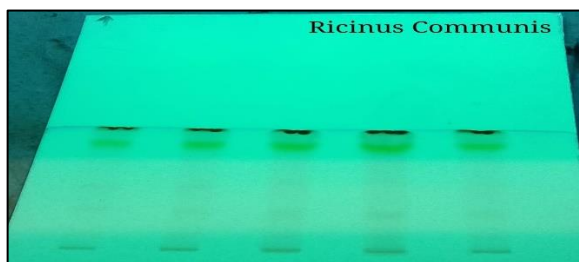


Fig no. 12 HPTLC of *Ricinus communis*



Wavelength of *Ricinus communis* extract

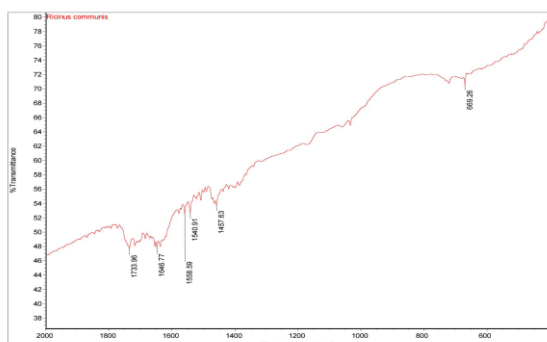


Figure no. 14

Discussion

The castor bean, or *Ricinus communis*, has long been used as a traditional herbal medicine for a number of different conditions. Its therapeutic properties are the main focus of this study, especially its possible cardioprotective effects against oxidative stress, which is a major contributing factor to cardiovascular diseases (CVD). Redox imbalance and oxidative stress aggravate a variety of heart-related conditions, such as myocardial infarction and hypertension (Cadeddu Dessalvi et al., 2021). Considering how common these ailments are becoming, investigating natural treatments like *Ricinus communis* may provide beneficial supplements to current treatments.

Conclusions

Ricinus communis exhibits great potential as a medicinal substance with cardioprotective and antioxidant qualities. Its scientific validity and historical use make it a viable option for additional study and use in the management of cardiovascular health. Further research should examine long-term impacts, the best dosage, and methods of action to completely clarify its advantages and safety in a range of demographics.

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