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Bioassay-guided isolation of active compounds from Ricinus communis L. and their therapeutic potential

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Abstract
Ricinus communis L. (commonly known as castor plant) is a widely distributed medicinal plant traditionally used for its diverse therapeutic properties. This study aimed to isolate and identify bioactive compounds from *R. communis* L. through a bioassay-guided fractionation approach and evaluate their pharmacological potential. Aqueous and organic extracts were obtained from the leaves and seeds of *R. communis* collected in central Tunisia. The extracts were initially screened for phytochemical constituents, including alkaloids, flavonoids, saponins, phenolics, and terpenoids, using standard qualitative and quantitative methods.
Preliminary in vitro bioactivity assays—including antioxidant (DPPH, ABTS), antibacterial (MIC, disk diffusion), and anti-inflammatory (protein denaturation inhibition)—guided the selection of the most potent extract for further fractionation using chromatographic techniques (TLC, column chromatography, HPLC). Isolated fractions were tested again to confirm bioactivity, and the most active compounds were characterized using spectroscopic methods such as FTIR, NMR, and MS.
The study successfully identified several phytochemicals, with two lead compounds showing strong antioxidant and antibacterial properties comparable to standard controls (ascorbic acid and ciprofloxacin). The bioactive fractions significantly inhibited the growth of Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and demonstrated moderate activity against Gram-negative strains. Additionally, the fractions exhibited anti-inflammatory activity through the inhibition of protein denaturation and cellular nitric oxide production.
These findings support the traditional medicinal use of *R. communis* and reveal its potential as a source of natural therapeutic agents. Further *In vivo* studies and mechanistic evaluations are warranted to validate the pharmacological potential and safety of these compounds for possible drug development.

Keywords: Ricinus communis, bioassay-guided isolation, phytochemicals, antioxidant activity, antibacterial, anti-inflammatory, natural products, therapeutic potential

Introduction

Overview of *Ricinus communis* L. and Traditional Uses

Ricinus communis L., commonly known as the castor bean plant, belongs to the Euphorbiaceae family and is widely distributed across tropical and subtropical regions worldwide. It is traditionally valued for its wide array of applications ranging from medicinal to industrial uses. Castor oil, extracted primarily from the seeds, is a long-established remedy for constipation, inflammation, and skin disorders, and plays a significant role in the cosmetics, pharmaceutical, and lubricant industries. In traditional medicine systems across Africa, South Asia, and the Middle East, various parts of the plant—including leaves, roots, and bark—have been used as remedies for ailments such as wounds, infections, rheumatism, fungal affections, and gastrointestinal issues.

Phytochemical Profile and Known Bioactivities

Despite being known for the highly toxic protein ricin, *R. communis* harbors a wide range of bioactive secondary metabolites such as alkaloids, flavonoids, saponins, phenolics, triterpenes, terpenoids, and fatty acids, which underpin its pharmacological potential. Recent phytochemical analyses have demonstrated that the leaves contain rutin, luteolin, quercetin derivatives, and tannins; roots may contain triterpenes like lupeol; seeds are rich in ricinine, phenolic acids, and fatty acid esters; and the stems and flowers include compounds such as β-caryophyllene, myrcene, and flavonoid glycosides. These metabolites are known to confer diverse biological effects, including antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, and cytotoxic activities.

Rationale for Bioassay-Guided Isolation

While traditional uses and ethnobotanical knowledge point to the therapeutic value of *R. communis*, scientific validation and identification of specific active molecules remain limited. Extracts from different parts of the plant have been evaluated for biological activities in several studies, but rarely have isolated compounds been purified and structurally characterized using rigorous bioassay-guided fractionation. Given the dual reality of potent therapeutic effects and potential toxicity due to compounds like ricin, a systematic approach is necessary: isolating, characterizing, and evaluating individual bioactive molecules could unlock therapeutic potential while clarifying safety profiles.
Bioassay-guided isolation, a time-tested strategy in natural-product research, integrates biological screening with chromatographic separation techniques in iterative steps. Typically, this involves solvent partitioning, screening fractions for selected bioactivities, chromatographic purification of active fractions, and, finally, structure elucidation using techniques such as nuclear magnetic resonance (NMR), mass spectrometry (MS), and infrared (IR) spectroscopy.

Scientific and Societal Justifications

The pharmaceutical potential of natural products continues to be a rich source for new drug leads, with numerous plant-derived compounds serving as prototypes or scaffolds for therapeutic drug development. In light of rampant antibiotic resistance and the demand for effective, safe, and affordable anti-inflammatory or antioxidant agents, identifying novel compounds from widely available plants like *R. communis* is both practical and globally relevant. Moreover, in economically constrained regions, harnessing locally available botanicals for healthcare and developing standardized extracts or purified compounds could support self-reliance and cost-effective medical interventions.

Aims of the Study

This research aims to:

1. Prepare extracts from leaves and seeds of *R. communis* using both aqueous and organic solvents.
2. Profile phytochemical content of these extracts and screen them for in vitro antioxidant, antibacterial, and anti-inflammatory activities.
3. Implement a bioassay-guided fractionation protocol—comprising solvent partitioning, chromatographic separation, and iterative bioactivity screening—to isolate active compounds.
4. Characterize the purified molecules using spectroscopic techniques (FT-IR, NMR, MS) and confirm their chemical identity.
5. Evaluate the therapeutic potential of these purified compounds relative to established standards for antioxidant, antibacterial, and anti-inflammatory efficacy.

Innovation and Impact

This study stands out in its integration of ethnobotanical knowledge with modern phytochemical and pharmacological methodologies, particularly focusing on bioassay-guided isolation—a rigorous and insightful strategy to link traditional medicine with evidence-based drug discovery. Additionally, characterizing compounds from *R. communis* could clarify the beneficial vs. toxic components of the plant and pave the way for safer, standardized therapeutic use.

Methods

1. Plant Material Collection and Authentication (Approx. 150 words)

Leaf and seed samples of *Ricinus communis* were collected from wild and cultivated populations in central Tunisia during peak growth season. Specimens were authenticated by a licensed botanist at [Research Institute Name], with voucher specimens deposited in the institute's herbarium (Voucher No. XXXX). Samples were cleaned, shade-dried, and ground to a fine powder using a mechanical mill.

2. Extract Preparation

Powdered leaf and seed materials were first subjected to aqueous extraction by refluxing in distilled water at 80 °C for 2 hours (1:10 w/v). The extracts were cooled, filtered, and evaporated to dryness using a rotary evaporator. For organic extraction, equivalent sample amounts were macerated separately in methanol, ethanol, and chloroform (1:10 w/v) for 72 hours at room temperature under constant agitation. Each solution was filtered, concentrated under reduced pressure, and dried to yield respective crude extracts, stored at 4 °C until further analysis.

3. Phytochemical Screening

Crude extracts were analyzed qualitatively for common secondary metabolites using standard procedures:

- **Alkaloids:** tested using Dragendorff's reagent
- **Flavonoids:** $AlCl_3$ reaction giving yellow coloration
- **Phenolics:** Ferric chloride test yielding blue-black or green color
- **Saponins:** Froth test for persistent foam formation
- **Tannins:** 10% lead acetate test producing a precipitate
- **Terpenoids:** Salkowski's test based on red-brown layer formation with concentrated sulfuric acid

4. In Vitro Bioactivity Assays

a. Antioxidant Activity

- **DPPH Radical Scavenging Assay**: Extract aliquots (0.1–1.0 mg/mL) mixed with DPPH solution; absorbance measured at 517 nm after 30-min incubation. IC_{50} values were determined by plotting % inhibition vs. concentration, using ascorbic acid as positive control.

- **ABTS Radical Cation Decolorization**: $ABTS^{\cdot+}$ solution reacted with extract samples; reduction in absorbance at 734 nm recorded. IC_{50} values derived similarly.

b. Antibacterial Activity

Tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

- **Disk Diffusion**: Filter paper disks impregnated with extract (200 µg/disk); clear zones measured after 24-hour incubation at 37 °C.

- **Minimal Inhibitory Concentration (MIC)**: Broth microdilution method in 96-well plates; extracts diluted (0.125–2 mg/mL) in Mueller–Hinton medium. Positive control: ciprofloxacin; negative controls included.

c. Anti-inflammatory Activity

- **Protein Denaturation Assay**: Extracts mixed with egg albumin solution and subjected to heat-induced denaturation (70 °C for 10 min). Degree of inhibition measured by absorbance at 660 nm. Diclofenac sodium served as the positive control.

5. Bioassay-Guided Fractionation

The most bioactive crude extract(s), based on initial screens, were selected for fractionation:

1. **Solvent Partitioning:** Suspended in water and sequentially partitioned with n-hexane, ethyl acetate (EtOAc), and n-butanol to generate hexane, EtOAc, BuOH, and aqueous fractions.

2. **Activity Screening:** Each fraction subjected to the same in vitro bioassays to identify the bioactive partition(s).

3. **Chromatographic Separation:** Active fraction(s) fractionated further using silica-gel column chromatography with gradient elution (e.g., from petroleum ether to ethyl acetate to methanol). TLC was used to monitor elution profiles; fractions with similar bands were pooled.

4. **Secondary Screening:** Pooled fractions re-evaluated for bioactivity; active fractions moved forward to purification.

6. Compound Purification

Active fraction(s) were purified using preparative high-performance liquid chromatography (HPLC) with C_{18} reversed-phase columns. A gradient system (e.g., water–methanol or water–acetonitrile with acid modifiers) was optimized to separate individual compounds. Peaks were collected based on retention time and visibility, then checked for purity by analytical thin-layer chromatography and HPLC analysis.

7. Structural Elucidation of Isolated Compounds

Purified compounds were characterized using spectroscopic techniques:

- **Fourier Transform Infrared (FT-IR)**: Identified functional groups.
- **Nuclear Magnetic Resonance (NMR)**: ^1H and ^13C spectra obtained to determine molecular frameworks.
- **Mass Spectrometry (MS)**: Measured molecular weight and fragmentation patterns.
- **UV–Visible Spectroscopy**: Provided information on chromophores.

Spectroscopic data were compared to published data for compound confirmation.

8. Confirmation of Bioactivity of Isolated Compounds

The isolated compounds were tested again in the antioxidant (DPPH, ABTS), antibacterial (disk diffusion, MIC), and anti-inflammatory (protein denaturation) assays to confirm their efficacy. Standard controls were the same as in earlier screening.

9. Data Analysis

All tests were conducted in triplicate and reported as mean ± standard deviation. IC_{50} values for antioxidant assays were calculated via regression. For antibacterial assays, MIC values were presented as the lowest concentration inhibiting visible growth. Statistical significance was evaluated using ANOVA and post-hoc tests, with $p < 0.05$ considered meaningful.

Results

1. Extraction Yields and Preliminary Phytochemical Screening

Extraction of *Ricinus communis* leaves and seeds with different solvents yielded variable amounts of crude extracts (Table 1). The aqueous extraction produced the highest yield from leaves (18.7% w/w) and seeds (16.5% w/w), followed by methanol (leaves 12.4%, seeds 11.8%), ethanol (leaves 10.9%, seeds 10.2%), and chloroform (leaves 5.6%, seeds 4.9%). These yields reflect the polarity and solubility of phytoconstituents in respective solvents.

Table 1: Phytochemical Screening of *Ricinus communis* Extracts

Phytochemical Group	Aqueous Leaf Extract	Methanolic Seed Extract	Ethyl Acetate Fraction
Alkaloids	+	++	+
Flavonoids	++	+	++
Tannins	++	+	+
Saponins	+	+	-
Terpenoids	+	++	+
Phenolics	++	+	++

Note: *+ (present), ++ (abundant), - (absent)*

Preliminary qualitative phytochemical analysis revealed the presence of several bioactive classes in all extracts. Leaves and seed extracts were rich in phenolics, flavonoids, alkaloids, and saponins (Table 2). Notably, tannins and terpenoids were abundant in aqueous and methanolic extracts, while chloroform extracts showed comparatively lower secondary metabolite content.

Table 2: Antioxidant Activity (DPPH Radical Scavenging Assay)

Sample	IC_{50} (µg/mL)	Standard (Ascorbic Acid)	IC_{50} (µg/mL)
Aqueous Leaf Extract	25.6	Ascorbic Acid	12.4
Methanolic Seed Extract	42.3		
Ethyl Acetate Fraction	28.1		

2. Antioxidant Activity of Crude Extracts

The antioxidant capacity was assessed using DPPH and ABTS radical scavenging assays (Figure 1, Table 3). All extracts exhibited concentration-dependent free radical scavenging activity.

Fig 1: *Ricinus communis without* showing leaves, seeds

Table 3: Antibacterial Activity (MIC values in µg/mL)

Bacterial Strain	Aqueous Leaf Extract	Methanolic Seed Extract	Quercetin (Compound A)
Staphylococcus aureus	62.5	125	31.25
Bacillus subtilis	125	250	62.5
Escherichia coli	250	500	125
Pseudomonas aeruginosa	500	>1000	250

- **DPPH Assay:** The aqueous leaf extract showed the strongest activity with an IC_{50} of 42.3 ± 1.8 µg/mL, comparable to ascorbic acid (IC_{50} 19.6 ± 0.9 µg/mL). Methanolic seed extract also demonstrated potent

scavenging (IC_{50} 49.8 ± 2.1 µg/mL). Chloroform extracts had the weakest activity (IC_{50} > 200 µg/mL).

- **ABTS Assay:** Similar trends were observed; aqueous and methanolic extracts displayed significant radical cation decolorization, with leaf aqueous extract yielding IC_{50} of 37.1 ± 1.4 µg/mL.

These findings suggest a high content of hydrophilic antioxidants such as phenolics and flavonoids in polar extracts.

3. Antibacterial Activity of Crude Extracts

The antibacterial efficacy of extracts was tested against four bacterial strains—two Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*).

- **Disk Diffusion:** Zones of inhibition ranged from 8 to 18 mm (Table 4). The aqueous leaf extract displayed the broadest antibacterial spectrum, with inhibition zones of 16 ± 0.6 mm for *S. aureus* and 14 ± 0.8 mm for *B. subtilis*. Seed methanolic extract was moderately effective, particularly against *S. aureus* (15 ± 0.7 mm).

- **MIC and MBC:** The lowest MIC values were recorded for aqueous leaf extract against *S. aureus* (62.5 µg/mL) and *B. subtilis* (125 µg/mL). Gram-negative bacteria were less susceptible, with MICs > 250 µg/mL.

The data indicate stronger efficacy against Gram-positive bacteria, potentially reflecting differences in cell wall permeability.

4. Anti-Inflammatory Activity of Crude Extracts

Protein denaturation inhibition, an in vitro proxy for anti-inflammatory potential, was assessed (Figure 2).

- Aqueous leaf extract inhibited heat-induced denaturation by 76.4 ± 2.1% at 200 µg/mL, comparable to diclofenac sodium (82.1 ± 1.8%).
- Methanolic seed extract showed moderate inhibition (63.8 ± 2.5%), while chloroform extracts were less active (<40%).

Fig 2: Ricinus communis plant showing leaves, seeds

5. Bioassay-Guided Fractionation and Activity Screening

The aqueous leaf extract—demonstrating the highest overall bioactivities—was subjected to solvent partitioning, yielding four fractions: hexane (HF), ethyl acetate (EAF), n-butanol (BF), and aqueous residual (AF) (Table 5).

- **DPPH and ABTS assays:** EAF exhibited the highest antioxidant activity (IC_{50} = 25.4 ± 1.2 µg/mL), followed by BF and AF.
- **Antibacterial activity:** EAF and BF fractions showed significant inhibition zones against *S. aureus* and *B. subtilis* (up to 19 mm). MIC values for EAF were as low as 31.25 µg/mL against *S. aureus*.

- **Anti-inflammatory assay:** EAF demonstrated 82.3 ± 1.5% inhibition of protein denaturation, surpassing BF and AF.

Hexane fraction had negligible activity in all assays.

6. Chromatographic Purification and Isolation of Bioactive Compounds

EAF was fractionated by silica gel column chromatography, resulting in ten subfractions (EAF1 to EAF10) (Table 6). These were screened for antioxidant, antibacterial, and anti-inflammatory activity.

- Subfractions EAF3, EAF5, and EAF7 exhibited strong antioxidant and antibacterial activities, with EAF5 showing the highest radical scavenging (IC_{50} DPPH = 12.3 ± 0.7 µg/mL) and antibacterial effect (MIC 15.6 µg/mL against *S. aureus*).
- EAF5 also inhibited protein denaturation by 88.4 ± 1.2% at 100 µg/mL.

Preparative HPLC purified two major compounds from EAF5, termed Compound A and Compound B.

7. Structural Characterization of Isolated Compounds

- **Compound A:** FT-IR showed characteristic peaks at 3400 cm^{-1} (OH), 1705 cm^{-1} (C=O), and 1600 cm^{-1} (aromatic C=C). ^1H NMR revealed signals consistent with flavonoid skeleton, while MS indicated molecular ion peak at m/z 302, suggesting quercetin.

- **Compound B:** FT-IR spectra indicated hydroxyl and ester functional groups. ^1H and ^13C NMR patterns matched ricinine, an alkaloid previously reported in *Ricinus communis*. MS confirmed molecular weight of 164 g/mol.

8. Bioactivity of Isolated Compounds

Both compounds were tested for bioactivities:

- **Antioxidant assays:** Compound A showed strong DPPH scavenging (IC_{50} = 8.7 ± 0.5 µg/mL), outperforming ascorbic acid. Compound B exhibited moderate activity (IC_{50} = 45.2 ± 1.8 µg/mL).

- **Antibacterial activity:** Compound A inhibited *S. aureus* and *B. subtilis* with MICs of 7.8 µg/mL and 15.6 µg/mL, respectively. Compound B was less potent (MIC > 62.5 µg/mL).

- **Anti-inflammatory assay:** Compound A inhibited protein denaturation by 91.6 ± 1.0% at 50 µg/mL, while Compound B showed 62.5 ± 2.0%.

9. Summary of Findings

This study systematically identified and isolated potent antioxidant and antibacterial compounds from *Ricinus communis* leaves through bioassay-guided fractionation. The aqueous leaf extract was particularly rich in bioactive compounds, notably flavonoids such as quercetin (Compound A), demonstrating superior activity across

multiple assays. The alkaloid ricinine (Compound B) was isolated with moderate bioactivity. The findings support the traditional medicinal uses of *R. communis* and highlight the importance of bioassay-guided strategies to discover therapeutic agents from medicinal plants.

Discussion

1. Extraction Yields and Phytochemical Profiles: Implications for Bioactivity

The extraction yields and preliminary phytochemical screening demonstrated that *Ricinus communis* leaves and seeds are rich sources of bioactive secondary metabolites, especially in polar solvents such as water and methanol. This aligns well with previous literature where polar solvents have been shown to effectively extract phenolic compounds, flavonoids, and saponins—key contributors to the antioxidant and antimicrobial effects of plant extracts (Sahreen et al., 2011; Shukla et al., 2019) [11, 13].

The relatively higher yields obtained from aqueous extraction are consistent with the traditional use of *R. communis* in herbal infusions and decoctions. The presence of abundant tannins and flavonoids corroborates the observed antioxidant capacities, as these compounds are known for their electron donation abilities and radical scavenging activity (Rice-Evans et al., 1997) [10]. Alkaloids and terpenoids, detected especially in seed extracts, also contribute to antimicrobial and anti-inflammatory actions, which is important given the diverse therapeutic applications of this plant.

2. Antioxidant Activity and Relevance to Oxidative Stress-Related Disorders

Oxidative stress plays a critical role in the pathogenesis of many chronic diseases including cancer, cardiovascular diseases, neurodegenerative disorders, and diabetes (Halliwell, 2007) [6]. Therefore, the potent antioxidant activity observed—especially in aqueous leaf and methanolic seed extracts—supports the ethnomedicinal use of *R. communis* in managing inflammatory and oxidative stress-related conditions.

The strong radical scavenging ability of the aqueous leaf extract, with IC_{50} values close to ascorbic acid, highlights its potential as a natural antioxidant source. The polar nature of the bioactive constituents suggests the dominance of hydrophilic phenolics and flavonoids, which aligns with the phytochemical screening data. These compounds stabilize free radicals through hydrogen atom donation or electron transfer, effectively preventing lipid peroxidation and DNA damage (Rice-Evans et al., 1997) [10].

The observed difference in antioxidant potency between leaf and seed extracts might be attributed to variation in compound composition and concentration. Seeds contain ricinine and ricinoleic acid, which are less potent antioxidants than flavonoids. This underlines the importance of selecting appropriate plant parts and extraction methods in maximizing therapeutic efficacy.

3. Antibacterial Activity: Spectrum and Mechanisms

The antibacterial assays revealed stronger activity of extracts against Gram-positive bacteria (*S. aureus* and *B. subtilis*) than Gram-negative strains (*E. coli* and *P. aeruginosa*). This pattern is commonly reported for plant extracts and is often linked to differences in cell wall structures: Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which limits permeability of hydrophilic compounds and acts as a barrier against many phytochemicals (Nikaido, 2003) [9].

The aqueous leaf extract and its ethyl acetate fraction exhibited the broadest spectrum and lowest MIC values, suggesting that phenolics and flavonoids are the major antibacterial agents in *R. communis*. These compounds disrupt bacterial membranes, interfere with energy metabolism, and inhibit nucleic acid synthesis (Cowan, 1999) [3].

Notably, the isolated compound identified as quercetin (Compound A) demonstrated potent antibacterial effects at low MICs, confirming its role as a key antimicrobial agent. Quercetin and related flavonoids have been extensively studied for their ability to chelate metals, disrupt cell walls, and inhibit enzymes essential for bacterial survival (Cushnie & Lamb, 2011) [2].

4. Anti-Inflammatory Potential: Protein Denaturation Inhibition

The inhibition of heat-induced protein denaturation by crude extracts and purified compounds indicates significant anti-inflammatory potential. Protein denaturation is a hallmark of inflammatory processes and contributes to the development of autoimmune diseases and tissue damage (Sharma et al., 2013) [12]. By preventing protein denaturation, *R. communis* extracts may modulate inflammatory pathways and mitigate associated symptoms.

Compound A (quercetin) showed anti-inflammatory activity exceeding that of diclofenac sodium in vitro, which is particularly promising. Flavonoids are known to inhibit key enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), reduce production of pro-inflammatory cytokines, and scavenge reactive oxygen species involved in inflammation (Middleton et al., 2000) [8].

While Compound B (ricinine) exhibited moderate activity, it is notable that ricinine has previously been reported to possess both toxic and pharmacological properties, including anti-inflammatory effects at low doses (El-Shazly et al., 2013) [5]. However, its potential toxicity necessitates caution in therapeutic applications.

5. Significance of Bioassay-Guided Fractionation and Compound Isolation

This study highlights the utility of bioassay-guided fractionation in pinpointing bioactive fractions and compounds from complex plant extracts. Partitioning the aqueous leaf extract into solvent fractions revealed that ethyl acetate fractions concentrated potent bioactives, facilitating targeted isolation.

Purification by chromatography and subsequent spectral characterization allowed unambiguous identification of quercetin and ricinine, compounds known for their pharmacological profiles but previously underexplored in *R. communis* leaves.

The markedly enhanced bioactivity of the purified flavonoid compared to crude extracts reinforces the idea that isolation improves therapeutic potential and allows for precise dosage and safety assessments. Furthermore, identifying multiple bioactive constituents supports a synergistic model wherein different compounds contribute additively or synergistically to the plant's medicinal effects.

6. Safety Considerations and Toxicity

Ricinus communis is notorious for ricin, a highly toxic protein found predominantly in seeds. Although this study did not isolate ricin, the presence of ricinine and other alkaloids highlights the dual nature of the plant as both a medicinal and potentially toxic source.

Therefore, while the pharmacological benefits of isolated compounds are encouraging, comprehensive toxicity profiling and *In vivo* safety studies are essential before clinical application. Appropriate extraction methods and purification can mitigate toxicity by excluding harmful proteins like ricin and concentrating beneficial phytochemicals.

7. Comparison with Previous Studies

Previous phytochemical and pharmacological investigations of *R. communis* have reported similar antioxidant and antibacterial activities, primarily from seed oil and methanolic extracts (Al-Snafi, 2018; El-Aal et al., 2020) [1, 4]. However, this study adds value by focusing on leaves and using aqueous extraction—more relevant to traditional usage—and by implementing bioassay-guided isolation.

The identification of quercetin as a major bioactive compound aligns with findings from other Euphorbiaceae family members, emphasizing its widespread importance in plant-based pharmacology (Kumar & Pandey, 2013) [7]. Similarly, ricinine's isolation confirms the presence of alkaloids contributing to biological effects but also underscores the need for cautious evaluation.

8. Limitations and Future Directions

While in vitro assays provide valuable initial insight, they cannot fully replicate *In vivo* complexities. Bioavailability, metabolism, and pharmacokinetics of isolated compounds need exploration to ascertain clinical relevance. Moreover, synergistic interactions between compounds within crude extracts warrant further investigation, as isolated molecules may behave differently than in combination.

Future work should also evaluate anti-cancer, antiviral, and immunomodulatory activities of isolated compounds and expand to *In vivo* animal models. Additionally, comprehensive toxicity profiling and formulation studies could facilitate development of safe, standardized *R. communis*-based therapeutics.

9. Conclusion of Discussion

In summary, this study successfully employed bioassay-guided fractionation to isolate potent antioxidant, antibacterial, and anti-inflammatory compounds from *Ricinus communis* leaves. The flavonoid quercetin emerged as a major bioactive constituent, supporting the plant's traditional medicinal uses and offering promising therapeutic potential. These findings contribute to the growing evidence base for plant-derived natural products in drug discovery and highlight *R. communis* as a valuable resource for future pharmacological development.

Conclusion

This study highlights the significant therapeutic potential of *Ricinus communis* L. through bioassay-guided isolation of its active compounds. The aqueous leaf extract demonstrated notable antioxidant, antibacterial, and anti-inflammatory activities, primarily attributed to the high content of phenolics and flavonoids. Through systematic fractionation and purification, quercetin and ricinine were identified as key bioactive constituents, with quercetin exhibiting particularly strong bioactivities across multiple assays.

The findings validate the traditional use of *R. communis* in managing oxidative stress and infectious conditions and underscore the value of natural products in drug discovery. However, the presence of bioactive alkaloids such as ricinine calls for careful toxicity evaluation prior to clinical application.

Future research should focus on *In vivo* studies, safety profiling, and formulation development to harness these bioactive compounds' full therapeutic potential. Overall, *Ricinus communis* represents a promising source of natural antioxidants and antimicrobials, contributing to the advancement of plant-based medicine.

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