

Antifungal Activity And Preliminary Test Of Phytochemical Screening Of Aqueous And Methanolic Extract Of Ricinus Communis L.

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Abstract: The bioactive compounds present in the plant are responsible for the medical properties of the plant. The present investigated is aimed in screening the bioactive compounds present in leaves of *Ricinus* communis L. an important ethnomedicinal plant. The qualitive analysis for the present phytochemicals was performed using methanolic and aqueous extracts of leaves. Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponin, tannin, and phenolics in all the extracts varying quantities. Since the plant contain high quantities of these new bioactive potential compounds. It is reliable to possess large number of pharmacological values like antifungal activities and are being employed for the treatment of different ailments in the indigenous systems of medicine.

Keywords: *Ricinus communis*; Methanolic extract; Phytochemicals screening; antifungal.

INTRODUCTION I.

Medicinal plants have been integral to healing and curing human diseases, primarily due to the presence of phytochemicals. These naturally occurring compounds in plant leaves, such as those found in *Ricinus* communis L. (castor oil plant), serve as defense mechanisms against various diseases and offer therapeutic benefits (Jena, and Gupta, 2012). The bioactive constituents, known as secondary metabolites, are the end products of primary metabolic processes including carbohydrate, amino acid, and lipid metabolism. Secondary metabolites, such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds, are vital for the medicinal properties of many plants. Identifying and isolating these active compounds is crucial for enhancing their therapeutic applications and ensuring the efficacy of plant-based remedies. R. communis L., belonging to the Euphorbiaceae family, is a well-known traditional remedy used globally for treating a wide range of diseases. The entire plant, including leaves, roots, stems, and fruits, has been utilized for its medicinal properties (Okhale, 2020). Various studies have confirmed the presence of diverse phytochemicals in R. communis, such as alkaloids, flavonoids, terpenes, and phenolic compounds including kaempferol, gallic acid, ricin, rutin, lupeol, ricinoleic acid, pinene, thujone, and gentisic acid. These compounds contribute to the plant's pharmacological activities, which include antifungal, anticancer, antimicrobial, insecticidal, antioxidant, antidiabetic, antinociceptive, anti-inflammatory, bone regenerative, analgesic, and anticonvulsant effects (Dada and Abioye, 2021). Recent studies have emphasized the antifungal properties of R. communis, particularly its methanolic leaf extracts, which have demonstrated efficacy against skin fungal pathogens, including species of Aspergillus. The plant also exhibits hepatoprotective effects and is traditionally used in treating skin fungal infections. The active components of castor oil, derived from R. communis, are believed to promote wound healing through their antioxidant activity and inhibition of lipid peroxidation (Patel, 2022). In this study, we validated the antimicrobial and fungicidal properties of R. communis using aqueous and methanolic extracts. Our findings align with earlier research, reinforcing the plant's potential as a valuable source of natural antifungal agents.

II. RESEARCH METHODOLOGY

3.1 Collection of plant material

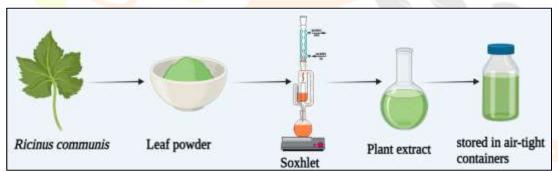
These wild medicinal plant (*Riccinus communis* L. (Arandi) were chosen for conducting the study on the basis of cost effectiveness, ease of availability and medicinal properties. Leaves of plants were collected from the campus of Krishi Vigyan Kendra (KVK) Banasthali Vidyapith, Jaipur, Rajasthan. Fresh leaves were collected washed thoroughly 2-3 times with running tap water and once with sterile distilled water remove adhering organic contaminants and then air dried at room temperature, dried samples were ground (Croma 500 W Mixer grinder *CRAK4184*) to make fine powder which was stored in air-tight containers for



further use (Sharma et al., 2022).

Figure 1. Photo plate indicating the test plants, their dried and grounded leaf powder sample of Ricinus communis L. (a-c)

3.2 Solvent extraction: The dried leaf powdered was performed to aqueous and methanolic extracts using the Soxhlet method for plant extracts. Twenty grams of leaf powdered was filled in the thimble and then successively extracted by aqueous and methanolic for 48 hours at 55°C. Using a rotary flash evaporator with reduced pressure, all of the solvent extracts were concentrated. Individual plant leaves were dried, and the leaf powder was utilized for solvent extraction by the Soxhlet method using aqueous and methanolic. The extracts were stored in airtight brown bottles until further use. The plant extracts were then used to



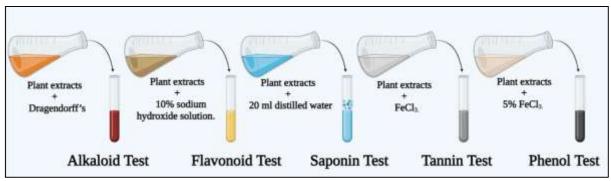
characterize the phytochemicals and test for antifungal efficacy (Singh et al., 2022).

Figure 2. Sampe preparation

- 3.3 Qualitative analysis of the plant extracts for phytoconstituents: The preliminary phytochemical analysis of the extracts carried out using aqueous and methanolic extracts and on the powdered specimen using standard procedure.
- **3.3.1 Test for alkaloids:** To test for the presence of alkaloids in the *Ricinus communis L*. leaf extracts, a few drops of the extract were stirred with 3 ml of 1% hydrochloric acid (HCl) on a steam bath. From this mixture, 1 ml was taken and divided into two separate test tubes. To one of the test tubes, a few drops of Dragendorff's reagent were added. The formation of an orange-red precipitate indicated a positive result for the presence of alkaloids in the extract (Harborne, 1998).
- **3.3.2 Test for flavonoids:** To test for the presence of flavonoids, a small amount of the plant extract was placed in a test tube. A few drops of 10% sodium hydroxide (NaOH) solution were then added to the extract. The development of a yellow color indicated the presence of flavonoids. To confirm this, dilute hydrochloric acid (HCl) was added; the disappearance of the yellow color upon the addition of the acid further confirmed the presence of flavonoids in the extract (Edeoga, 2005).
- **3.3.3Test for saponins:** To test for the presence of saponins, a few drops of the plant extract were added to 5 ml of distilled water in a test tube. The mixture was then shaken vigorously and subsequently warmed. The formation of stable foam was observed, which indicated the presence of saponins in the extract (Obadoni and Ochuko, 2001).
- **3.3.4 Test for tannin:** To test for the presence of tannins, about 2 ml of the plant extract was mixed with 2 ml of distilled water in a test tube. A few drops of ferric chloride (FeCl₃) solution were then added to the

mixture. The formation of a green precipitate indicated the presence of tannins in the extract (Change et al., 2002).

3.3.5 Test for phenols: To detect the presence of phenolic compounds, 500 mg of the plant extract was dissolved in 5 ml of distilled water. To this solution, a few drops of neutral 5% ferric chloride (FeCl₃) solution were added. The appearance of a dark green color indicated the presence of phenolic compounds



in the extract (Hagerman and Butler, 1989).

Figure 3. Phytochemical screening analysis of medicinal plant Ricinus communis L.

3.4 Thin layer chromatography

The aqueous and methanolic extracts were applied as spots using capillary tubes onto one end of a thin-layer chromatography (TLC) plate, positioned approximately 1 cm above the edge. After air drying, the plate was placed in a beaker containing a solvent mixture of ethyl acetate and methanolic in a 6:4 ratio. The samples were allowed to migrate toward the other end of the plate. Once the solvent front had reached the desired height, the TLC plate was removed and air-dried. A 2% ninhydrin solution was then sprayed onto the plate, followed by an additional air-drying period of 10 minutes. The plate was subsequently visualized under UV light, revealing violet-coloured spots on the plate, indicating the presence of compounds in the extracts.

3.5 Antifungal assay

The study utilized the fungal strains *Aspergillus niger* to assess the antifungal activity of plant extracts using the well diffusion method according to a standard protocol. For the preparation of the stock solution, 2 mg of the plant extract was dissolved in 1 ml of 2% dimethyl sulfoxide (DMSO) in a sterile tube. Sabouraud dextrose agar was used as the growth medium, and the fungal spores were uniformly spread across the surface of the agar plates with a sterile cotton swab. The plates were then incubated for 15 minutes at room temperature. Wells with a diameter of 5 mm were punched into the agar for drug loading. aqueous and methanolic extracts of the plant were prepared, dissolved in DMSO, and then labelled accordingly. Each well was loaded with 10 μ l or 20 μ l of the plant extracts using a micro-pipette. The plates were incubated at 27 ± 2°C for 7 days. After incubation, the fungal growth on each plate was measured, and the diameter of the zones of inhibition was recorded to evaluate the antifungal efficacy of the plant extracts.

III. RESULTS AND DISCUSSION

The curative properties of *R. communis* plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, saponin, tannin, and phenolics.

Phytochemical analysis: The phytochemicals analysis of the aqueous and methanolic extracts from the leaves of *R. communis* is shown in Figure 4 and Table 1 respectively. Moreover, Table 1 and Figure 4 indicate the different phytochemicals compounds of *R. communis*. The methanolic extracts from the leaves of *R. communis* plant contained a number of phytochemicals such as alkaloids, flavonoids, saponin, tannin, and phenolics. Tannins and saponin are present in leaves extracts. Flavonoids are present in methanolic extract better then aqueous extracts. Terpenoids and steroids were absent in both extracts. Saponin have the properties of wound healing. Alkalis are used in medicine for reducing acne and fungal infection they are attributed for antifungal activity. *R. communis* could be a good candidate for discovering novel complementary drugs. Further experimental and advanced clinical studies are required to explore the pharmaceutical, beneficial therapeutic and safety prospects of *R. communis* with its phytochemicals as an herbal and complementary medicine for combating various diseases and disorders.

Table 1. Phytochemical analysis of aqueous and methanolic extracts from leaves of Ricinus communis.

Phytochemical	Phytochemical	Aqueous	Methanolic
compounds	test		
Test for alkaloids	Dragendorff's test	Positive	Positive
Test for flavonoids	Lead Acetate test	Positive	Positive
Test for saponin	Foam test	Positive	Positive
Test for tannin	Ferric Chloride test	Positive	Positive
Test for phenolics	Ferric Chloride test	Positive	Positive
Test for steroids		Negative	Negative
Test for carbohydrates		Negative	Negative

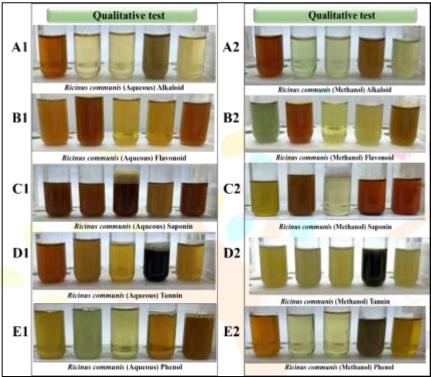


Figure 4. Photo plates showing the presence of extraction of phytochemicals (bioactive compounds) associated with the test plants (qualitative) *Ricinus communis*: (A1:A2) presence of alkaloids; (B1:B2) presence of flavonoids; (C1:C2) presence of saponin; (D1:D2) presence of tannin; (E1:22) presence of phenol respectively.

Thin layer chromatography: Compound identification was performed using silica gel-coated thin-layer chromatography (TLC) on methanolic and ethyl acetate extracts. Upon visualization under visible light, a



light violet colour was observed on the tracks of the TLC plate, indicating the presence of a compound in the sample.

Figure 5. Photo plates show thin layer chromatography: (A) aqueous extract (B) methanolic extract Antifungal assay: This data corroborated the findings of other authors where these compounds exhibited antifungal activity. The antifungal activity of *R. communis* was assessed using plant extracts prepared in dimethyl sulfoxide (DMSO). The aqueous and methanolic extract of *R. communis* demonstrated significant antifungal activity against *A. niger*, with the maximum inhibition zones observed at a concentration of 20µl dilutions of the plant extract. The growth inhibition was attributed to the active compounds in the aqueous and methanolic extract and DMSO. In contrast, the methanolic extract of *R. communis* showed better

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antifungal activity than aqueous extract against *A. niger*. The largest inhibition zone, measuring 18mm, was recorded with the methanolic extract and 15mm, was recorded with the aqueous extract of *R. communis* against *A. niger respectively*.

Table 2. Antifungal activity of aqueous and methanolic extracts from leaves of *Ricinus communis*.

Plant Extracts	Fungal Culture	Zone of inhibition
Aqueous	Aspergillus niger	15
Methanolic		18

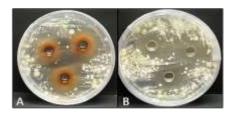


Figure 6. Photo plates show antifungal activity: (A) aqueous extract (B) methanolic extract of R. communis plant extract against A. niger.

CONCLUSION

Ricinus communis L. is a medicinal plant with diverse pharmacological applications, offering potential treatments for various diseases and disorders. Its antifungal activities are particularly noteworthy, providing a promising approach to combating life-threatening infections worldwide. This plant is a rich source of secondary metabolites, including alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, and reducing sugars, all of which contribute to its medicinal properties.

The methanolic plant extract of *R. communis* was found to actively impeding with fungal at varying inhibitory levels. The methanolic plant extract of *R. communis* were observed to be more active against the fungal species used in the assay. The therapeutic potential of *R. communis* is evident in both its aqueous and methanolic extracts, as these forms contain bioactive compounds responsible for its pharmacological effects. Further studies focusing on the isolation and characterization of these compounds could elucidate their mechanisms of action, paving the way for the development of novel drugs. By conducting in vitro and animal studies, researchers can explore how these phytochemicals interact with biological targets, leading to the design of new therapeutic agents. This study provides compelling evidence that solvent extracts of *R. communis* contain bioactive compounds with significant medicinal value, justifying its traditional use in treating various diseases. Moving forward, our priority will be the purification, identification, and characterization of these bioactive compounds to fully realize their therapeutic potential.

IV. ACKNOWLEDGMENT

The authors are grateful to Professor Ina Aditya Shastri, Vice-Chancellor, Banasthali Vidyapith, Rajasthan. We also provide thanks to DST-CURIE for providing financial assistance for conducting our research work.

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