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Research Article

Qualitative Phytochemicals Analysis and *In vitro* Antibacterial Efficacy of Methanolic and Hydroalcoholic *Ricinus communis* L. Leaves Extracts

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Abstract

The prevalence of multidrug-resistant (MDR) bugs has evolved as a significant global health concern worldwide. The escalating prevalence of multidrug resistant (MDR) pathogens is concerning, emphasizing the significance of developing novel and innovative antimicrobial therapies. The use of plant-based pharmaceuticals is increasingly growing as a safer, simple, affordable and chemical free substitute. Consequently, numerous research laboratories are actively engaged in the exploration of novel biologically active compounds with antimicrobial potential. *Ricinus communis* is one such important medicinal herb renowned for its significant amounts of flavonoids, phenols and tannins etc. The antibacterial efficacy of methanol and hydro-alcohol crude leaves concentrates of *R. communis* was determined against *P. vulgaris* (MTCC 1771), *S. pyogenes* (MTCC 442), *B. subtilus* (MTCC 441), *S. aureus* (MTCC 96) using agar well diffusion assay. Keeping in view the potential of *Ricinus communis*, the current research aimed to investigate, qualitative phytochemicals assessment and antimicrobial efficacy. Therefore, this study reveals that *R. communis* extracts contain a variety of chemical components linked to their antimicrobial properties giving a maximum Zone of Inhibition (ZOI) by Methanolic concentrate in comparison to the Hydro-alcoholic extract.

Keywords: *Ricinus communis*; Phytochemicals; Multidrug Resistance; Zone of Inhibition; *P. vulgaris; S. pyogenes; B. subtilus; S. aureus*; ZOI

Introduction

Emergence of drug-resistant bacteria and the frequency of zoonotic pathogenic microbes have exacerbated the severity of infectious diseases [1]. As a consequence of antibiotic resistance, numerous surveillance programs are being put in place globally. These initiative programs have demonstrated that, with regional variations, the emergence of antibiotic resistance among different infectious agents to both identical and distinct pharmaceuticals appears to be growing over time [2]. Antibiotic resistance has substantially devastated both human and animal health, leading to prolonged hospitalizations and escalating healthcare expenses [3]. Other pharmaceuticals are currently evaluated based on a broad spectrum of plant sources in order to determine their pharmacological capabilities, as secondary metabolites exhibit minimal adverse reactions to pharmaceuticals, resistance, and leftovers [1].

Modern drugs that have been developed as a result of medicinal plants research include ephedrine, artemisinin, topotecan, theobromide, digitoxin, 3-n-butylphthalide, huperzine, vinblastine, anisodamine, teniposide, acetyldigoxin, and Tu [1,4]. The sole species of the monotypic genus Ricinus is *Ricinus communis* L., which has been categorised as belonging tothe Euphorbiaceae family. Local terms used for the plant include "Qobboo" in Afan Oromo, "Gulo" in Amharic, and "Castor oil plant and castor bean plant" in English. This plant species thrives across a wide altitude range, spanning elevations of 400 to 4500 meters above sea level, itis found in temperate as well as tropical regions worldwide. Ricinus plant features a hollow stem with 15 cm thick leaves and is capable of attaining heights of 5–10m on a perennial basis. Its long, alternate, stipulated leaves typically exhibit dark green or reddish hues, while its fruit is encased in a thorny covering that protects the seed. It has been

reproduced by employing a combination of both self-pollination and outcrossing via wind (entomophily) or insect (entomophily) pollination [5].

In Ethiopia, *R. communis* is utilized for treating diarrhoea, actinomycosis, blackleg, wound and skin dermatitis/rashes, and mastitis in cattle [1]. Researchers in Pakistan and Ghana, evaluated the antibacterial efficacy of *R. communis* extracts from leaves utilizing different solvents, and significant antimicrobial characteristics were demonstrated by Methanolic extract [6,7]. Plant species are not entirely influenced by the plant's medicinal capabilities but elevation, illumination, moisture temperature, and most significantly seasonal variations plays the vital role in influencing its capabilities. Distinct active phyto-constituents and their concentrations have additionally been influenced by variations in the geographical distribution of medicinal plant species [8]. Globally, multiple herbs are explored therapeutically for generating potent and safer therapeutic agents. Diverse signs of applications of *R. communis* plant's efficacy in the management of numerous ailments have generated interest concerning thisscientific research. The current research investigation highlights the *in vitro* antibacterial activities of methanolic and hydro-alcoholic concentrates of *R. communis* leaves against some pathogenic bacterial species.

Materials and Methods

Plant material

Fresh and young leaves of *R. communis* have been picked from Rampur Bushahar, located in District Shimla of Himachal Pradesh, India. The identification as well as verification of the plant was conducted by Botany Department, of Himachal Pradesh University Shimla.

Plant's processing

The plant leaves were properly cleansed using distilled water and allowed to shade dry for few days. The dried leaves of *R. communis* were thoroughly crushed employing an electrical blender and preserved within an airtight container for future utilization. In accordance to solvent's rising polarity, the plant material was incorporated to the solvent in a ratio of 1:10 and recovered over the course of three consecutive days utilizing different solvents. Following the separation of the extracts, the methanolic and hydroalcoholic filtrates were allowed to dry at room temperature with Whatman's No. 1 filteration paper. The dried extract was diluted by using 10% DMSO, and further stored in airtight jar at 4°C until further investigation [9].

Qualitative analysis

The Preliminary screening of plant phytoconstituents was conducted in order to determine a profile of methanolic and hydroalcoholic extracts for its chemical composition. To detect the presence of various phytoconstituents in extracts, following tests were performed [10].

Test for tannins and phenols

The crude extract has been treated with 2mL concentration of 2% solution of $FeCl_3$. The levels of phenols and tannins in the sample were assessed by its black or blue-green coloration [11].

Tests for flavonoids

Alkaline reagent test

The plant concentrate was allowed to mix in 2mL of 2% NaOH solution. On adding few drops ofdilute acetic acid, the originally developed bright or intense yellow colour of the solution becomes colorless, confirming the presence of flavonoid in the plant sample [11].

Shinoda test (Magnesium hydrochloride ribbion test)

Crude plant material was gradually mixed with potent HCl drop by drop, followed by addition of a few magnesium ribbon fragments. After a few minutes, a pink scarlet color developed confirms the flavonoid presence [12].

Test for saponins

The plant extract is allowed to shake briskly along with 5 millilitres of distilled water in a tube. Appearance of consistent foam interpreted as an indication of saponins presence [10].

Test for glycosides

Salkowski's test

The plant concentrate was allowed to mix to 2 millilitres of chloroform. Further, the test tube was filled with 2mL highly concentrated sulphuric acid and shaken carefully. Presence ofglycone component of glycoside or a steroidal ring, was confirmed by the presence of reddish- brown colour [10].

Liebermann's test

The plant concentrate was mixed in 2mL chloroform along with 2mL acetic acid. The entire mixture was allowed to cool on ice. A quantitative concentration of sulphuric acid was employed. Change in color from violet to deep blue and from deep blue to green demonstrated the evidence for the existence of steroidal nucleus, or glycoside's glycone portion [12].

Keller-kilani test

The crude concentrate was allowed to mix in 2mL glacial acetic acid and a few drops of freshly prepared 2% $FeCl_3$. The liquid mixture was then transferred to an additional testing tube containing 2mL of concentrated sulfuric acid. A brown colored ring formed within the interphaseregion depicted the existence of cardiac glycosides within the sample [10].

Test for steroids

To the Crude plant extract 2mL of chloroform was added along with concentrated H_2SO_4 side by side. The presence of steroids could be seen by the presence of red coloration within the lower chloroform layer. An intense red coloration formed with in the lower chloroform layer confirmedthe existence of steroids. Other test for the presence of steroids was carried when plant extract was mixed to 2 millilitres chloroform. The reaction mixture was further treated with 2mL of concentrated H_2SO_4 followed by adding acetic acid. Greenish coloration in the concentrate depicted the presence of steroids [11].

Test for terpenoids

2mL chloroform was allowed to mix with plant's crude extract and made to evaporate and dry completely. Followed by the addition of 2mL concentrated H_2SO_4 to this mixture a rough heating was allowed for 2 minutes. The presence of terpenoids was revealed by its grayish color [11].

Test for alkaloids

The crude plant concentrate was dissolved in 2mL of 1% sulphuric acid and allowed to heat slowly. Mayer's reagent as well as Wagner's reagent were subsequently incorporated within the reaction mixture. The appearance of turbidity within the precipitate formed was marked as a confirmatory result for alkaloids [11,13].

Test for microorganism

The test bacterial species employed in the present investigation were *P. vulgaris* (MTCC 1771), *S. pyogenes* (MTCC 442), *B. subtilus* (MTCC 441) and *S. aureus* (MTCC 96). Initially, all four bacterial strains were kept for overnight incubation at 37°C temperature, further being sub- cultured in Muller- hinton broth (Hi-Media).

Positive and negative control

As a positive control, 1 mg/mL of chloramphenicol was utilized to evaluate the bacterial strains. The negative control was 10% DMSO.

Antibacterial activity assay

Antibacterial efficacy of methanolic and hydro-alcoholic extracts of *Ricinus communis* was tested using the agar-well diffusion approach [14]. Initially, bacterial strains were cultured in Muller-Hinton broth overnight, followed by centrifugation for 10 minutes at 10,000 rpm at room temperature. After removing the supernatant from the solution, the cell pellet was then dissolved in 10mL normal saline and sample's optical density (O.D.) was measured at 595nm. The obtained (O.D.) for all bacterial cultures was adjusted to 0.4 using normal saline. Bacteria with the desired (O.D.) were further used for spreading in the agar well diffusion experiment [15]. Various concentrations of the *R. communis* extracts were utilized to establish a correlation between extract activity and dosage. Muller-Hinton agar medium was prepared by dissolving 3.8g of Muller-Hinton agar in 100 milliliters distilled water with pH 7.0, followed by autoclavingof mixture and cooling it to 45°C. Petri dishes were filled with 25mL of seeded Muller-Hinton agar and left to solidify, after which a sterile borer was employed to punch the well. To these wells approximately 100 mg/mL stock solution was filled, and made to stand for 2 hours at normal room temperature before incubation at 37°C. Control experiments were carried out simultaneously, in order to utilize the respective solvent extracts to fill in the wells. After 24 hours of incubation, the plates were examined, and the antibacterial efficacy of extracts was assessed by measuring its zone of inhibition in millimeters. Final outcomes of the findings were analysed and compared to those of chloramphenicol at 1 mg/mL concentration. Experiment research was conducted in triplicates and the data was subjected to statistical analysis forcalculating mean ± SD.

Results

Phytochemical screening of plant extracts

The therapeutic aspects of medicinal plants can possibly be attributed primarily to the abundance of numerous secondary metabolites present in the various plant parts. Subsequent leaves extracts from the *Ricinus communis* plant revealed a diverse range of phytocostituents such as glycosides,flavonoids, saponins, alkaloids, terpenoids, steroids, phenols and tannins (Table 1). Therefore, preliminary qualitative screening procedures may be advantageous in detecting bioactive constituents and their progression. The phytochemical investigation of *Ricinus communis* might contribute to the discovery of novel drugs.

Antimicrobial screening of crude methanolic and hydro-alcoholic extracts

Numerous researchers have reported the medicinal benefits of *Ricinus communis* plant asa therapeutic agent. Based on agar

Phytoconstituents	Pet-ether	Acetone	Chloroform	Ethanol	Methanol	Hydro-alcoholic	Aqueous
Glycosides	++	-	-	+	++	++	+
Flavonoids	-	-	-	-	-	-	-
Saponins	-	+	-	-	+	++	+
Alkaloids	+	+	-	-	+	-	-
Terpenoids	+	+	-	+	+	-	-
Steroids	+	+	-	+	+	-	-
Phenols and Tannins	-	-	-	-	++	+	+

Table 1: Phytochemical screening of *Ricinus communis* leaves extracts.

well diffusion methodology, antibacterial potency of methanolic and hydro-alcoholic leaf extracts of Castor oil plant has been evaluated against four major pathogenic bacterial strains (Figure 1). Antibacterial efficacy of the methanolic and hydroalcoholic leaves concentrate demonstrated analogous to those of standard antibiotics (chloramphenicol).

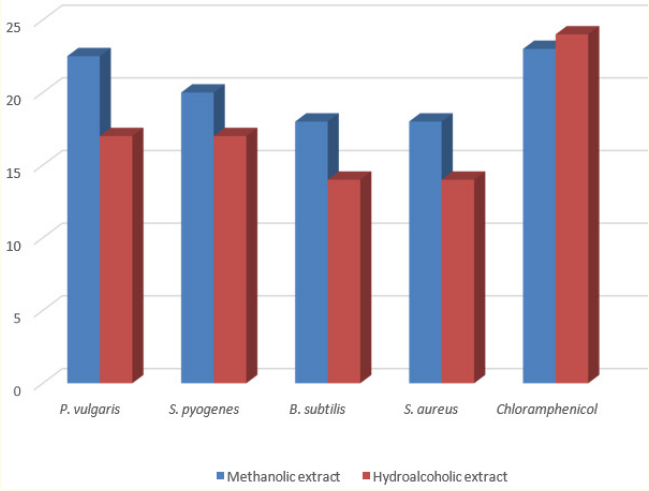

Figure 1: Antibacterial profiling of Methanolic and Hydro-alcoholic leaves extract against pathogenic micro-organisms.

Methanolic leaves extract of *Ricinus communis* found to be efficacious against all four pathogenic bacterial strains at a concentration of 100mg/mL and showed significant antimicrobial activity. The extract exhibited maximum zone of inhibition (ZOI) against *P. vulgaris* (22.5 ± 0.56) followed by *S. pyogenes* (20 ± 0.78). The antimicrobial activity is less but significant in the case of *B. subtilis* and *S. aureus* with a (ZOI) of (18 ± 0.56).

The Hydroalcoholic leaf extract showed a maximum (ZOI) of (17 ± 0.24) against *P. vulgaris* and (17 ± 0.41) against *S. pyogenes*. However, against *B. subtilis* and *S. aureus* the (ZOI) was same i.e. (14 ± 0.27). Chloramphenicol, is used as a positive control and exhibited (ZOI) of (23 ± 0.29)and (24 ± 0.64) against methanolic and hydroalcoholic extracts respectively. In treatment with DMSO as a negative control, no zone of inhibition was encountered among both extracts.

Bacterial Isolates	Zone of Inhibition (mm)Methanol (100mg/ml)	Zone of Inhibition (mm) Hydro-alcoholic (100mg/ml)
P. vulgaris (MTCC 1771)	22.5 ± 0.56	17 ± 0.24
S. pyogenes (MTCC 442)	20 ± 0.78	17 ± 0.41
B. subtilis (MTCC 441)	18 ± 0.56	14 ± 0.27
S. aureus (MTCC 96)	18 ± 0.56	14 ± 0.27
Positive control (Chloramphenicol)	23 ± 0.29	24 ± 0.64
Negative control (DMSO)	No zone	No zone

Table 2: Sensitivity of the test microorganisms to methanolic and hydro-alcoholic leaves extract of *Ricinus communis.*

Discussion

In the present research, phytochemical efficaciousness and antibacterial effectiveness of *R. communis* methanolic and hydro-alcoholic crude leaf extracts have been assessed. An initial step towards generating novel and potent pharmaceuticals is demonstrated through *in vitro* assessment of antibacterial potential of plant extracts. The results of the current research demonstrate that the methanolic concentrate displayed the highest antibacterial potential, while the hydro-alcohalolic concentrate displayed lowest effectiveness against all four pathogenic microorganisms: *P. vulgaris*, *S. pyogenes*, *B. subtilis*, and *S. aureus*. These outcomes are in accordance with other investigations, which revealed that a

majority of antibacterial compounds in plants are readily soluble in methanol [16,17]. Since, drug-resistance has been evolving promptly [18] and medical professionals are encountering significant challenges in handling an array of infectious illnesses [19], these kinds of plants deserve to be assigned all possible opportunities to offer with their inherent antimicrobial benefits. Actual components exhibiting antibacterial capabilities needs thus, be extracted and further characterized. In-depth research needs to be carried out on the appropriate levels and harmful consequences associated with these novel medications upon humans as well as animals. This study validates the medicinal plant's potential as a good pharmaceuticals.

Conclusion

The castor oil plant was gathered from Rampur Bushahar, District Shimla, Himachal Pradesh. Crude plant extracts yielded a wide range of secondary compounds. Plant extracts were qualitatively investigated in order to search for existing plant components. Maximum zone of inhibition demonstrates the antibacterial profiling of plant extract. Plant-derived crude extracts were examined for their capability to combat pathogenic bacterial cultures such as *P. vulgaris*, *S.pyogenes*, *B. subtilis*, and *S. aureus*. Significant antibacterial efficacy is demonstrated by the plant extracts, putting them on scale when compared to tested and currently utilized pharmaceutical drugs. The results of this investigation, also suggest that it may be possible to separate a potential lead chemical out of these therapeutic plants, which might be utilized as a base for developing potent antibacterial agents. Therefore, further investigations on clinical efficacy trials, safety, toxicity, and affordability evaluations must be instigated promptly, in order to get to the conclusion of generating precursor compounds towards novel potent antimicrobialdrugs.

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Conflict of Interest

The authors possess no conflict of interest.

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