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Original Article

Microbiology Section

Antibacterial Activity of *Ricinus communis* Extracts against Common Human Pathogens Obtained from Surgical Wound Infections in a Tertiary Care Hospital of Semi-urban Set-up at Andhra Pradesh, India

ARCHANA VOLETI[1], SRIUSHASWINI BANDARU[2], KVSB VIDYA SAGAR[3], RANGA RAO ACHANTA[4], BANADARU NARASINGA RAO[5]

ABSTRACT

Introduction: A number of medicinal compounds are manufactured with great exactness and simply from easily available raw materials at normal temperature and pressure from highly sophisticated chemical factories called plants and its parts. There are many methods of research associated with the medicinal plants and traditional medicine has shown very little attention in modern research and less effort has been done to upgrade the practice of using medicinal plants.

Aim: To assess the antibacterial activity of *Ricinus communis* leaf extracts against common human pathogens obtained from surgical wound infections in a tertiary care teaching hospital.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology of a Tertiary Care teaching Hospital for a period of one month in January 2021. The pathogenic bacteria isolated from the surgical wounds were used for this study and the antibacterial activity of medicinal herb leaf extract *Ricinus communis* L. was investigated against gram positive bacteria and gram negative bacteria. Antimicrobial susceptibility testing was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disc diffusion technique using the extract, ampicillin as positive control and Dimethyl Sulphoxide (DMSO) as negative control. The zones of inhibition were measured using a special measuring scale. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the medicinal plant were also tested against the same pathogenic bacterial strains that were isolated. The statistical analysis of frequency and mean were calculated and the results were tabulated.

Results: The methanol crude extract of plant leaves (300 mg/mL) showed significant zone of inhibition against gram positive and gram negative bacteria. Comparatively, very less zone of inhibition was obtained from the aqueous unrefined extract of medicinal plant when compared to the methanol solvent extracts. DMSO was taken as negative control and no zone of inhibition was seen. Ampicillin (10 µg) was taken as positive control and has shown significant zone of inhibition against the isolated bacterial pathogens from wound infections. The best MBC value was observed in the methanol extract and in aqueous extract, the growth was extensive and full.

Conclusion: *Ricinus communis* concentrated methanol leaf extract had shown effective antibacterial activity in comparison with the standard ampicillin. Based on further chemical, pharmacological and molecular studies in the future on this leaf extract and identify phytochemical constituents in the leaf, seed, stem, roots and to screen other potential bioactivities of the leaf extract may be recommended for the treatment of wounds.

Keywords: Disc diffusion, Kirby-Bauer, Minimum bactericidal concentration, Minimum inhibitory concentration

INTRODUCTION

The richest organic compounds are found in the plant kingdom, and they have been regularly used since ancient times for therapeutic purposes. A numerous unrefined drugs that are isolated from these medicinal herbs have greatest implicational ability to treat a wide variety of diseases and *Ricinus communis* holds an extraordinary position in this group of medicinal plants [1,2]. *Ricinus communis* belongs to the family Euphorbiaceae that is a group of flowering plants. The most important plant parts of *Ricinus* that are utilised for treatment are the root, stem, leaves, fruits, whole of aerial parts, the complete plant and flowers [3]. The plant is proven to contain antioxidant properties in its methanolic leaf extract anti-inflammatory activity, anti-diabetic activity and antibacterial activity [4-7]. The plant has hepatoprotective effect and is used in the treatment of skin cancer [8,9].

The parts of these medicinal healing herbs have depicted their therapeutic value and have been used to avert, alleviate or heal several human ailments. Since, ancient times in terms of traditions that are related to herbal medicine, India is one of the leading countries in Asia and it includes a large number of plant species which includes Ayurveda, Unani, Siddha, and Tibetan [10]. In today's scenario, there has been a live again momentum of attention in healing plants as the awareness about the insufficient ability of manufactured pharmaceutical products to control major human ailments and there is a real need to discover new compounds from the medicinal herbs. The therapeutic potential of these medicinal plants is boundless in the treatment of infectious diseases and also minimises the adverse effects that are often associated with synthetic antimicrobials. There has been a realistic medicinal effect of these plant materials that has result from the combinations of secondary products present in the plant that are secondary metabolites such as steroids, tannins, alkaloids, and phenolic compounds, flavonoids, resins and gums that produce a significant physiological action on body. It was evident that these healing plants and their products have been used to heal diarrhoea, dysentery, cough, cold, cholera, fever, bronchitis and in healing of wounds [11]. Therefore, the present study was done to assess the antibacterial activity of *Ricinus communis* leaf

extracts against common human pathogens obtained from surgical wound infections.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology of a Tertiary Care teaching Hospital for a period of one month in January 2021. Institutional Ethics Committee (IEC) approval for this study was obtained (GVPIHCMT/IEC/20210204/04) and the study was carried out in various stages at Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India.

Inclusion criteria: Isolates obtained from only surgical wounds. Organisms included for study: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa.*

Exclusion criteria: Wound infections (prick infections, diabetic foot, burn wounds and accident wounds etc.,) were not included in the study. The patients with antibiotic history in the recent past and also on topical application of antimicrobial ointments were excluded from the study. Other isolates like *Acinetobacter, Citrobacter, Enterobacter* etc., were not included in the present study.

Study Procedure

Collection of *Ricinus communis* leaves: The *Ricinus communis* plants used for the present study were collected in the hospital campus and the leaves were used for the present study during January 2021. Its botanical identity was confirmed from the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Preparation of extracts by methanol and aqueous solvents: *Ricinus communis* leaves were washed and cleaned with distilled water and were air dried at room temperature for 10 days. Using a blender the leaves were grinded into a fine powder. Hundred grams of the finely churned plant materials was taken in 1 Liter methanol and aqueous extract by soaking the powder at room temperature for 48 hour. Using whatman filter paper No. 42 the extracts were filtered and concentrated using evaporator, warmed on water bath at 70°C for the aqueous extract and temperature of 50°C for methanol extracts, to obtain semi-solid products.

Collection of bacterial cultures: The bacterial cultures were obtained by isolation of pathogenic bacteria from the clinical specimens obtained from the surgical units, Department of Surgery, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India. Various isolates were obtained but the present study was done using one gram positive bacteria- *Staphylococcus aureus* and other gram negative bacteria- *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*. No patient was directly involved in this study as the study material obtained from the test requisition for pus culture and sensitivity. These bacterial cultures were sub-cultured on nutrient agar gel and stored at 4°C.

Inoculum preparation for antimicrobial sensitivity testing: A loopful of bacterial colony was suspended in 5 mL of nutrient broth and incubated at 37°C for 4-6 hours till desired turbidity was obtained. As per the microbial culture and sensitivity guidelines and the turbidity was matched with 0.5 McFarland standard [12].

Preparation of disc for antibacterial activity by Kirby-Bauer disc diffusion method: Discs of 6 mm diameter were prepared using sterile Whatmann No.1 filter paper [13]. The *Ricinus* leaf extracts (100 mg/mL, 200 mg/mL and 300 mg/mL) obtained using methanol and aqueous extract was mixed with 1 mL of 5% DMSO. The discs were impregnated with 20 µL of different concentrations of the extract to check their antibacterial activity. Ampicillin disc (10 µg) was used as positive control and the 5% DMSO disc was used as a negative control.

Disc diffusion method: Disc diffusion method was done as proposed by Kirby-Bauer where the antibacterial activity of *Ricinus* leaf extract was determined [13]. Mueller Hinton agar of about 15 mL was poured in sterile petriplates and was allowed to solidify. Dried plates were taken and 0.1 mL of 0.5 MacFarland standardised inoculum suspension was poured uniformly and was spread with the help of sterilised cotton swab. The cotton swab was allowed to collect the excess inoculum to be drained and the plates were set to dry for 5 minutes. Using sterile forceps the discs with extract concentration in the methanol solvent and aqueous solvent were placed on the surface of the agar plate separately and gently pressed to ensure contact with the surface of media. A 5% DMSO was used as a negative control and the ampicillin (10 µg/disc) was used as positive control in the present study. Then the plates were incubated at 37°C overnight and the zone of inhibition was measured by a special measuring scale obtained from HiMedia Mumbai. These tests were done in triplicate for statistical relevance and the average was taken as the final result.

MIC for bacteria: As described by Ericsson HM and Sherris JC, the MIC of the medicinal plant extracts was tested against the isolated bacteria on Mueller Hinton broth by broth macro dilution method [14]. The *Ricinus* leaf extract was dissolved in 5% DMSO to obtain 320 mg/mL stock solution. 0.5 mL of stock solution was incorporated into 0.5 mL of Mueller Hinton broth for bacteria to get a concentration of 320, 160, 80, 40 and 20 mg/mL for *Ricinus* leaf extract and 50 µL of standardised suspension of the test organism was transferred on to each tube. Only the organism without leaf extract was taken as control and the tubes were incubated at 37°C for 24 hours. The lowest concentration at which no growth of tested organism appeared after macroscopic evaluation was determined as MIC [15].

MBC: This test was conducted by sub-culturing 50 µL from each dilution well where no growth was observed. The minimum concentration of the *Ricinus* leaf extract that has no growth on sub-culturing was taken as MBC [16]. The degree to which the medium has retarded light based on opacity was measured at a wavelength of 655 nm of turbid meter after 24 hours from each well. The experiment was repeated three times.

STATISTICAL ANALYSIS

Frequency and mean were measured and the results were finalised and tabulated.

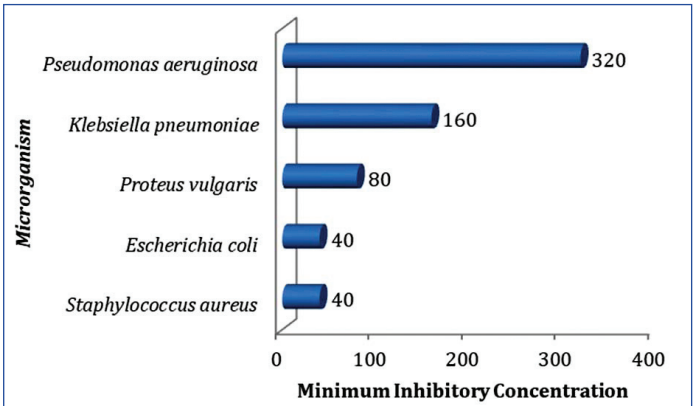
RESULTS

The crude extract of *Ricinus communis* leafs in methanol solvent with 300 mg/mL showed highest zone of inhibition against *Staphylococcus aureus* (25±0.2 mm) and was very minimal for *Pseudomonas aeruginosa* (11±0.3 mm). Other gram negative bacteria that were tested with 300 mg/mL had significantly shown the sensitive zones of inhibition. *Escherichia coli* (22±0.2 mm), *Proteus vulgaris* (21±0.2 mm), *Klebsiella pneumoniae* showed (20±0.3 mm). No zone of inhibition was seen in DMSO that was taken as negative control. The zone of inhibition for positive control ampicillin (10 µg) has shown the zone size ranging from 12±0.3 mm to 24±0.8 mm against the tested bacterial pathogens [Table/Fig-1]. The aqueous extract had not shown any significant zones of inhibition hence the results were not tabulated.

S. No.	Microrganism	Methanol (mg/mL concentration)			Ampicillin 10 µg/disc	DMSO 5%
		100	200	300		
1.	*Staphylococcus aureus*	19±0.3	21±0.8	25±0.2	24±0.8	Nil
2.	*Escherichia coli*	15±0.0	16±0.5	22±0.2	21±0.5	Nil
3.	*Proteus vulgaris*	11±0.3	12±0.1	21±0.2	20±0.3	Nil
4.	*Klebsiella pneumoniae*	11±0.3	12±0.2	20±0.3	19±0.6	Nil
5.	*Pseudomonas aeruginosa*	16±0.6	18±0.5	11±0.3	12±0.3	Nil

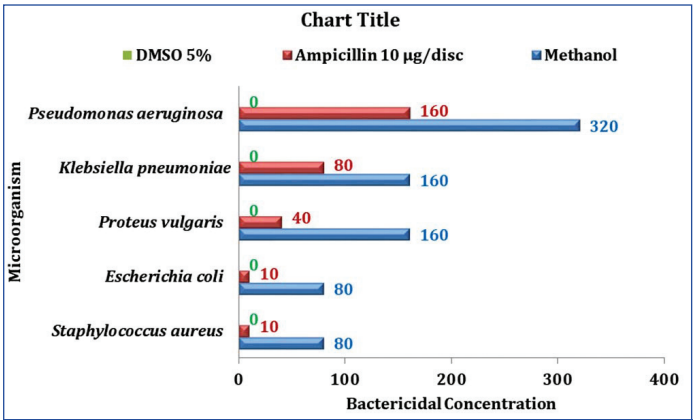
[Table/Fig-1]: Antibacterial activity of leaf extracts of *Ricinus communis*.

The results of *Ricinus communis* leaf extract MIC value against isolated pathogenic bacteria from wound infections is tabulated as the tested range was chosen between 20 mg/mL to 320 mg/mL [Table/Fig-2]. This procedure was done using only methanol leaf extract and the readings were recorded against the isolated pathogens. No inhibition was observed for *Pseudomonas aeruginosa* even at 320 mg/mL. Hence, the average MIC was (220 mg/mL).



[Table/Fig-2]: Minimum Inhibitory Concentration (MIC) mg/mL of leaf extracts of *Ricinus communis*.

The MBC of the medicinal leaf extract of *Ricinus communis* were tested against various bacterial isolates and the results were showed in [Table/Fig-3]. The best MBC was recorded with methanol leaf extract of *Ricinus communis* (300 mg/mL). This concentration has shown the highest mean zone of inhibition against *Staphylococcus aureus* (80 mg/mL) and gram negative bacilli *Escherichia coli* (80 mg/mL) followed by *Proteus vulgaris* (160 mg/mL) *Klebsiella pneumoniae* (160 mg/mL). No zone of inhibition was noticed in *Pseudomonas aeruginosa* (320 mg/mL) with the dilution used for testing. As negative control no zone of inhibition was seen.



[Table/Fig-3]: Minimum Bactericidal Concentration (MBC) mg/mL of leaf extracts of *Ricinus communis*.

DISCUSSION

In this present study, the antibacterial activity of *Ricinus communis* leaf extract was tested against the common human pathogens that were isolated from the surgical wounds. This showed that methanol solvent extract at 300 mg/mL concentration has shown the maximum activity. This inference is also supported by a study that was done by Naz R and Bano A on antimicrobial potential of *Ricinus communis* leaf extracts in different solvents against pathogenic bacterial and fungal strains in which they concluded that the efficient activity of *Ricinus communis* leaf extract in methanol solvent has shown significant potential to inhibit the pathogenic bacteria [16].

Another study was done by Hajrah N et al., where the anti-bacterial activity of *Ricinus communis* L. against bacterial pathogens *Escherichia coli* and *Klebsiella oxytoca* was evaluated by Transmission Electron Microscopy (TEM) [17]. In their study, they observed that the leaf extract had significant inhibition on the pathogenic bacteria they opted for the study. As the present study was conducted, similarly the leaf extract of *Ricinus* was evaluated for its antibacterial activity by agar well diffusion method. Leaf extract at 2 mg/mL dose showed a zone inhibition of 18 mm against *Escherichia coli* and a zone of 17 mm inhibition were shown by *Klebsiella oxytoca* that was significantly sensitive. However, the antibacterial activity was least against *Staphylococcus aureus*. This study has supported the present study showing an evidence of phytochemical antimicrobial activity of *Ricinus communis* leaf extract but, this study was done with 300 mg/mL. Well organised clinical research is being conducted on *Ricinus* which may provide promising results on therapeutic use of this plant and its parts in the future. At various concentration the results obtained were different [18]. Based on further chemical, pharmacological and molecular studies in the future on this leaf extract and identify phytochemical constituents in the leaf, seed, stem, roots, and to screen other potential bioactivities of the leaf extract may be recommended for the treatment of wounds [19-23].

Limitation(s)

The present study was limited to testing of 100 mg/mL, 200 mg/mL and 300 mg/mL of *Ricinus* leaves identified. Testing of different dilutions and testing with other species of *Ricinus* were limitation of the study.

CONCLUSION(S)

This observational study suggests that the simplest aliphatic methyl alcohol solvent extraction was satisfactory to verify the antibacterial properties of *Ricinus communis* leaf extract. This study also supports the traditional system of medicine which says that plant and its parts are used to treat various infectious diseases. This study also encourages cultivation of these plants that are highly precious in large scale to increase the economic status of the under developed sectors and cultivators in the country.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India.
2. Assistant Professor, Department of Surgery, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India.
3. Tutor, Department of Microbiology, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India.
4. Professor, Department of Surgery, Gayatri Vidya Parlshad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India.
5. Professor and Head, Department of Microbiology, Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Mrs. Archana Voleti,
GF 1, Venu Abode, Durganagar, Chandrampalem, Madhurwada,
Visakhapatnam-530041, Andhra Pradesh, India.
E-mail: archana.cartor@gmail.com

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