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ANTIBACTERIAL ACTIVITY OF *Ricinus communis* L. SOLVENT EXTRACTS AGAINST PATHOGENIC BACTERIA

S. Parimala* and D. Sangeetha,
Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.

Abstract

Plant cells are highly sophisticated chemical factories where a large variety of chemical compounds are manufactured with great precision and ease from simple raw materials at normal temperature and pressure. Plants are thus a very important renewable source of raw materials for the production of a large variety of chemicals. In the present study, the antibacterial activity of medicinal plant leaf extract *Ricinus communis* L. was investigated against bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Enterococcus epidermis, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Proteus vulgaris, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri* and *Enterobacter aerogenes*). The methanol crude extract of plant leaves (300 mg/ml) showed zone of inhibition against the Gram positive and Gram negative bacteria. The zone of inhibition obtained from the aqueous crude extract of medicinal plant against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control. The positive control Ampicillin (20 µg) showed zone of inhibition against the tested bacterial pathogens.The Minimum inhibitory concentration (MIC) value of the medicinal plant was tested bacteria. The Minimum bactericidal concentration (MBC) of the medicinal plant was tested against various bacterial isolates. The best MBC value was recorded in the methanol extract at 600 nm when compared to other solvent extracts. Maximum bacterial growth was recorded against the aqueous extract.

Key words: *Ricinus communis*, Antibacterial activity, Disc diffusion test, MIC and MBC.

1. Introduction

India has a rich heritage of knowledge on plant based antimicrobial drugs for use in preventive and curative medicine against various infectious diseases. Many countries in this universe like India are very much suited for development of drugs from medicinal plant. Because of its vase and wide variations in soil and climate, the Indian sub – continent is suitable for cultivation of large number of medicinal and aromatic plant which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavour and food and agrochemical industries. A large number of medicinal plants grow wild and exploited especially for use in indigenous pharmaceutical houses. Some of these plants produce valuable drugs which have high export potential (Rathish *et al*., 2005).

Developing a medicinal plants sector, across the various states of India has become an important issue in present scenario. There are many aspects of research associated with the medicinal plants sector. The significant contribution to the society, traditional medicine has experienced very little attention in modern

Corresponding author*: **S. Parimala
E. mail: pkmparimala42@gmail.com
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research and development and less effort has been done to upgrade the practice (Giday *et al*., 2003). Medicinal plants offer alternative remedies with enormous opportunities to produce income, employment and foreign exchange for under developing countries. Many traditional healing herbs and their parts have been shows its medicinal value and it can be used to prevent, alleviate or cure several human ailments. India is one of the leading countries in Asia in terms of the wealth of traditional knowledge systems related to herbal medicine and it employs a large number of plant species which includes Ayurveda, Siddha, Unani and Tibetan. Nowadays, there has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects a recognition of the validity of many traditional claims regarding the value of natural products in health care.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body. Compounds extracted from different parts of the plants can be used to cure diarrhoea, dysentery, cough, cold, cholera, fever, bronchitis, etc.

2. Materials and Methods

Collection of *Ricinus communis* leaves

The leaves of *Ricinus communis* was collected from Chidambaram, Cuddalore district, Tamil Nadu, India during February 2014 and the collected plants were used for the present research.

Collection of bacterial cultures

Twelve different bacterial cultures of Gram positive and Gram negative bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. Gram positive bacteria - *Staphylococcus aureus* (MTCC - 6908), *Streptococcus pyogenes* (MTCC - 1925), *Streptococcus pneumoniae* (MTCC - 5542) and *Enterococcus epidermis* (MTCC - 8765). Gram negative bacteria - *Escherichia coli* (MTCC - 1258), *Proteus vulgaris* (MTCC - 3310), *Pseudomonas aeruginosa* (MTCC - 1748), *Klebsiella pneumoniae* (MTCC - 3384), *Vibrio cholerae* (MTCC - 4865), *Salmonella typhi* (MTCC - 9280), *Shigella flexneri* (MTCC - 2770) and *Enterobacter aerogenes* (MTCC - 2729).

Maintenance of bacterial cultures

The bacterial cultures were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4 °C.

Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of organisms in 5 ml of Nutrient broth and incubated at 37 °C for 12 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standard and then used for the determination of antibacterial activity.

Preparation of disc for antibacterial activity

Six millimeter diameter discs were prepared using sterile Whatmann No.1 filter paper. The medicinal plant crude extracts (100 mg/ml, 200 mg/ml and 300 mg/ml) obtained using solvents (Aqueous, Chloroform, Methanol,

Ethanol and Acetone) were mixed with 1 ml of 5 % Dimethyl sulfoxide (DMSO). The discs were impregnated with 20 µl of different solvent extracts to check their antibacterial activity. The Ampicillin (20 µg) was used as positive control and the 5 % DMSO was used as a negative control.

Disc diffusion method

The antibacterial activity of medicinal plant extracts were determined by Disc diffusion method proposed by Bauer *et al.* (1966). Twenty ml of sterilized Mueller Hinton agar was poured in sterile petriplates and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded with the help of sterilized cotton swab. The excess inoculum was drained and the plates were allowed to dry for five minutes. After drying, the discs with extract were placed on the surface of the plate by using sterile forceps and gently pressed to ensure contact with the agar surface. The Ampicillin (20 µg/disc) was used as positive control and the 5% DMSO was used as a negative control in these assays. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated for three times for concordance results.

Minimum inhibitory concentration for bacteria

Minimum inhibitory concentrations (MIC) of the medicinal plant extracts against bacterial isolates were tested in Mueller Hinton broth by Broth macro dilution method (Ericsson and Sherri, 1971). The medicinal plant extracts were dissolved in 5% DMSO to obtain 640 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 640, 320, 160, 80, 40 and 20 mg/ml for medicinal plant extracts and 50 µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of medicinal plant extracts. The culture tubes were incubated at 37^{o}C for 24 hours. The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing 50 µl from each well showing no apparent growth. Least concentration of medicinal plant extract showing no visible growth on sub-culturing was taken as Minimum Bactericidal Concentration (MBC). The optical density of each well was measured at a wavelength of 655 nm after 24 hours. All experiments were performed in duplicate and repeated three times.

3. Results and Discussion

The antibacterial activity of medicinal plant leaf extracts of *Ricinus communis* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table – 1 and 2. The methanol crude extract of *Ricinus communis* (300 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Staphylococcus aureus* (25 mm) and *Pseudomonas aeruginosa* (25 mm) followed by *Streptococcus pyogenes* (24 mm), *Enterobacter aerogenes* (24 mm), *Klebsiella pneumoniae* (24 mm), *Enterococcus epidermis* (24 mm), *Proteus vulgaris* (24 mm) and *Streptococcus pneumoniae* (24 mm). Minimum zone of inhibition was noticed in *Vibrio cholerae* (23 mm), *Escherichia coli* (23 mm), *Salmonella typhi* (23 mm) and *Shigella flexneri* (23 mm). The minimum zone of inhibition obtained from the aqueous extract of the medicinal plant *Ricinus communis* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO blind control. The positive control Ampicillin (20 µg) showed zone of inhibition was ranging from 23 ± 0.8 mm to 29 ± 0.8 mm against the tested bacterial pathogens.

Ajayi and Akintola (2010) screened the leave extracts from medicinal plants *in vitro* in the laboratory for their antibacterial activity against

two prominent enteric bacteria, *Escherichia coli* and *Salmonella typhimurium* using the agar disc diffusion method. The tyndalized leave extract of *C. zambesicus* showing antibacterial inhibition zone of 4 and 2 mm against *Salmonella typhimurium* and *Escherichia coli* exhibited highest activity than the autoclaved samples and other plant sources tested independently or combined, showing that the combinations of the extract samples do not exhibit synergistic effects.

Sivasakthi *et al*. (2011) evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Datura metel* against nine pathogenic bacterial isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Datura metel* ethanol extract was tested by using Agar well diffusion method. The ethanol extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (26 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (8 mm). The ethyl acetate extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (19 mm) against *Escherichia coli*. There was no zone of inhibition against *Pseudomonas aeruginosa*.

Saranraj and Sivasakthivelan (2012) tested the antibacterial activity of *Phyllanthus amarus* was tested against Urinary tract infection causing bacterial isolates *viz*., *Staphylococcus aureus, Serratia marcescens, Escherichia coli, Enterobacter* sp., *Streptococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa*. The *Phyllanthus amarus* was shade dried and the antimicrobial principles were extracted with methanol, acetone, chloroform, petroleum ether and hexane. The antibacterial activity of *Phyllanthus amarus* was determined by Agar Well Diffusion Method. It was found that methanol extract of *Phyllanthus amarus* showed more inhibitory activity against UTI causing bacterial pathogens when compared to other solvent extracts.

The Minimum inhibitory concentration (MIC) value of *Ricinus communis* against tested bacteria was ranged between 10 mg/ml to 320 mg/ml and the results were showed in Table - 3. The lowest Minimum inhibitory concentration (MIC) (1.25 µg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Enterococcus epidermis, Proteus vulgaris* and *Escherichia coli.* The Minimum bactericidal concentration (MBC) of the medicinal plant *Ricinus communis* was tested against various bacterial isolates and the results were showed in Table - 4. The best Minimum bactericidal concentration (MBC) value was recorded in the methanol extract of *Ricinus communis* when compared to other solvent extracts. Nkere and Iroegbu (2005) studied the MIC values of the ethanol extracts of root and stem bark of *Picarlima nitida* reported the MIC values ranging from 6.25 to 50 mg/ml. Okoli and Iroegbu (2005), from their studies reported the MIC value in the range of 3.125 to 12.50 mg/ml for the ethanol root extracts of *Synclisa scabrida*.

4. Conclusion

The present observations suggests that the organic solvent extraction was suitable to verify the antibacterial properties of medicinal plant *Ricinus communis* and they supported by many investigation. The present study justifies the claimed uses of leaves in the traditional system of medicine to treat various infectious diseases caused by the bacteria and fungi. This present study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators in the country. The obtained results may provide a support to use of the plant in traditional medicine. Based on this further chemical and pharmacological investigations can be done to isolate and identify minor chemical constituents in the seeds and to screen other potential bioactivities may be recommended.

Table - 1: Antibacterial activity of crude extracts of *Ricinus communis*

Concentration of the *Ricinus communis* extracts (mg/ml) and Zone of inhibition (mm)											
S. No	**Micro organisms**	**Methanol**			**Ethanol**			**Acetone**			**Positive Control***
		100	**200**	**300**	**100**	**200**	**300**	**100**	**200**	**300**	
1.	*Staphylococcus aureus*	19 ± 0.3	21 ± 0.8	23 ± 0.2	16 ± 0.3	17 ± 0.1	18 ± 0.2	13 ± 0.2	14 ± 0.3	16 ± 0.2	27 ± 0.5
2.	*Streptococcus pyogenes*	18 ± 0.2	19 ± 0.4	20 ± 0.3	15 ± 0.2	16 ± 0.2	17 ± 0.3	13 ± 0.1	14 ± 0.2	15 ± 0.4	23 ± 0.2
3.	*Pseudomonas aeruginosa*	16 ± 0.6	18 ± 0.5	21 ± 0.3	14 ± 0.2	15 ± 0.3	16 ± 0.2	12 ± 0.3	13 ± 0.2	14 ± 0.3	27 ± 0.6
4.	*Enterobacter aerogenes*	15 ± 0.0	16 ± 0.5	17 ± 0.4	13 ± 0.2	14 ± 0.3	15 ± 0.2	11 ± 0.2	12 ± 0.3	13 ± 0.2	28 ± 0.4
5.	*Klebsiella pneumoniae*	11 ± 0.3	12 ± 0.2	14 ± 0.3	10 ± 0.2	10 ± 0.4	11 ± 0.2	9 ± 0.3	9 ± 0.8	10 ± 0.3	29 ± 0.4
6.	*Enterococcus epidermis*	13 ± 0.0	13 ± 0.4	15 ± 0.2	11 ± 0.2	12 ± 0.3	13 ± 0.2	10 ± 0.2	11 ± 0.2	11 ± 0.4	25 ± 0.3
7.	*Proteus vulgaris*	11 ± 0.3	12 ± 0.1	14 ± 0.2	9 ± 0.7	10 ± 0.6	11 ± 0.3	8 ± 0.2	9 ± 0.4	9 ± 0.8	25 ± 0.2
8.	*Streptococcus pneumoniae*	13 ± 0.2	14 ± 0.3	16 ± 0.2	11 ± 0.4	12 ± 0.6	13 ± 0.3	10 ± 0.8	11 ± 0.6	12 ± 0.1	22 ± 0.6
9.	*Vibrio cholerae*	13 ± 0.0	13 ± 0.4	15 ± 0.3	11 ± 0.2	12 ± 0.2	13 ± 0.2	10 ± 0.2	10 ± 0.6	11 ± 0.3	23 ± 0.5
10.	*Escherichia coli*	15 ± 0.0	16 ± 0.5	17 ± 0.2	13 ± 0.2	14 ± 0.2	15 ± 0.0	11 ± 0.2	12 ± 0.2	13 ± 0.2	25 ± 0.4
11.	*Salmonella typhi*	10 ± 0.3	11 ± 0.2	13 ± 0.1	9 ± 0.2	9 ± 0.8	10 ± 0.3	8 ± 0.1	9 ± 0.3	9 ± 0.6	20 ± 0.2
12.	*Shigella flexneri*	9 ± 0.5	10 ± 0.6	11 ± 0.1	8 ± 0.3	8 ± 0.6	9 ± 0.5	7 ± 0.3	8 ± 0.2	8 ± 0.6	24 ± 03

*Ampicillin (20 µg)

Table - 2: Antibacterial Activity Of Crude Extracts Of *Ricinus communis*

Concentration of the *Ricinus communis* extracts (mg/ml) and Zone of inhibition (mm)								
S. No	**Micro organisms**	**Chloroform**			**Aqueous**			**Positive Control***
		100	**200**	**300**	**100**	**200**	**300**	
1.	*Staphylococcus aureus*	11 ± 0.3	12 ± 0.6	13 ± 0.4	10 ± 0.4	11 ± 0.2	11 ± 0.4	27 ± 0.5
2.	*Streptococcus pyogenes*	10 ± 0.6	11 ± 0.4	12 ± 0.3	9 ± 0.5	10 ± 0.4	10 ± 0.6	23 ± 0.2
3.	*Pseudomonas aeruginosa*	11 ± 0.2	11 ± 0.6	12 ± 0.8	10 ± 0.4	11 ± 0.2	11 ± 0.3	27 ± 0.6
4.	*Enterobacter aerogenes*	10 ± 0.6	11 ± 0.2	11 ± 0.4	9 ± 0.6	10 ± 0.2	10 ± 0.4	28 ± 0.4
5.	*Klebsiella pneumoniae*	8 ± 0.4	9 ± 0.2	9 ± 0.6	7 ± 0.2	7 ± 0.6	8 ± 0.5	29 ± 0.4
6.	*Enterococcus epidermis*	9 ± 0.6	10 ± 0.1	10 ± 0.4	8 ± 0.4	9 ± 0.2	9 ± 0.5	25 ± 0.3
7.	*Proteus vulgaris*	7 ± 0.4	8 ± 0.2	8 ± 0.6	6 ± 0.6	7 ± 0.2	7 ± 0.5	25 ± 0.2
8.	*Streptococcus pneumoniae*	9 ± 0.4	10 ± 0.2	10 ± 0.6	8 ± 0.2	8 ± 0.4	9 ± 0.3	22 ± 0.6
9.	*Vibrio cholerae*	9 ± 0.3	9 ± 0.6	10 ± 0.3	8 ± 0.6	9 ± 0.2	9 ± 0.5	23 ± 0.5
10.	*Escherichia coli*	9 ± 0.4	10 ± 0.6	11 ± 0.4	8 ± 0.4	9 ± 0.1	9 ± 0.3	25 ± 0.4
11.	*Salmonella typhi*	7 ± 0.2	7 ± 0.6	8 ± 0.3	6 ± 0.3	7 ± 0.4	7 ± 0.6	20 ± 0.2
12.	*Shigella flexneri*	6 ± 0.4	7 ± 0.2	7 ± 0.4	6 ± 0.5	7 ± 0.1	7 ± 0.4	24 ± 03

*Ampicillin (20 µg) Mean ± SD

Table – 3: Minimum inhibitory concentration of crude extracts of *Ricinus communis*

Minimum inhibitory concentration (MIC) of *Ricinus communis* (mg/ml)						
Microorganisms	**Aqueous**	**Chloroform**	**Ethanol**	**Acetone**	**Methanol**	**Positive Control***
Staphylococcus aureus	160	20	40	80	10	20
Streptococcus pyogenes	80	40	80	40	10	20
Pseudomonas aeruginosa	160	20	40	80	20	20
Enterobacter aerogenes	320	80	80	40	40	40
Klebsiella pneumonia	320	40	80	40	20	40
Enterococcus epidermis	320	40	80	40	20	20
Proteus vulgaris	160	40	80	80	40	20
Streptococcus pneumoniae	320	40	80	160	20	20
Vibrio cholerae	320	40	80	160	20	40
Escherichia coli	160	20	40	40	10	40
Salmonella typhi	160	40	80	80	40	20
Shigella flexneri	160	40	80	80	40	20

*Ampicillin (20 μg)

Table – 4: Minimum bactericidal concentration of crude extracts of *Ricinus communis*

Minimum bactericidal concentration (MBC) of *Ricinus communis*						
Microorganisms	**Aqueous**	**Chloroform**	**Ethanol**	**Acetone**	**Methanol**	**Positive Control***
Staphylococcus aureus	320	40	80	160	20	40
Streptococcus pyogenes	160	80	160	80	20	40
Pseudomonas aeruginosa	320	40	80	160	40	40
Enterobacter aerogenes	640	160	160	80	80	80
Klebsiella pneumoniae	640	80	160	80	40	80
Enterococcus epidermis	640	80	160	80	40	40
Proteus vulgaris	320	80	160	160	80	40
Streptococcus pneumoniae	640	80	160	320	40	80
Vibrio cholerae	640	80	160	320	40	40
Escherichia coli	320	40	80	80	20	40
Salmonella typhi	320	80	160	160	80	40
Shigella flexneri	320	80	160	160	80	40

*Ampicillin (20 μg)

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