International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Inhibitory Effect of Various Extracts of *Ricinnus Communis* on Human Pathogens

Mitter C. S.

Department of Zoology, Sathaye College, Vile-Parle (E), Mumbai, 400 057, Maharashtra, India

Abstract: ***Extracts of Riccinus communis were made in mid-polar solvent acetone and non-polar solvent, petroleum ether by using fresh stem, leaves and tender fruits. Bioassay and Minimum Inhibitory Concentration (MIC) was done against various gram positive and gram negative bacteria by agar cup method. Significant activity was found in all acetone extracts against mainly gram positive bacteria and some gram negative bacteria. MIC of all potent extracts was at 0.5 mg/ml against most of the susceptible microorganisms.***

Keywords: *Riccinus communis*, bioassay, gram positive, gram negative bacteria, MIC.

1. Introduction

Man has been using plants as medicines for cure and management of diseases from prehistoric times. Natural products can provide a clue to the synthesis of new antimicrobial chemicals. Natural chemicals are relatively safe to man and his environment.

The World Health Organization has also realized that an effective health agenda for developing countries can be achieved by alternative medicines including traditional herbal medicine and has advised and urged developing countries of the world to utilize their medicinal plant resources to achieve the goal of primary health care **[1].** Among the entire flora 35,000 to 70,000 species have been used for medicinal purposes. The resistance to antibiotics and high recurrence rates are the serious health threats associated with various infections. Many bacteria are present in the environment of hospitals and majority of them are resistant to ampicillin and co-trimoxazole **[2]**. Various phytochemicals are well known for their antimicrobial properties and can be of great significance in therapeutic treatments. Antibacterial, antifungal and antioxidant activity of various plant species were studied by many workers **[3], [4], [5], [6]** and **[7].**

2. Literature Survey:

R. communis, commonly known as castor plants, treat constipation, skin ulcers, eye irritations, some gynecological infections, restore hair. It is used as ideal lubricating oil in industrial equipments. The castor bean plant is native to India, where it is commonly called 'Erand' **[8].** In India it is being used extensively for all types of gastrointestinal problems like constipation, dysentery and inflammatory bowel disease. It is also used to treat bladder and vaginal infections and asthma. Seed carnel (hull) boiled in milk and water and taken internally to relieve arthritis and lower back pain accompanied by sciatica. In the Canary Islands poultices made from leaves of castor bean plant is useful to increase milk secretion and relieve inflammation and milk stagnation in the mammary glands, if applied on nursing mother. Applying the poultice to the abdominal area promotes normal menstruation. It also enhances the immune system, and is effective in AIDS (T. cell count increases). It also helps in eliminating chronic problems with epilepsy, hypersensitivity, liver and gall bladder diseases and chronic fluid retention. It also prevents abdominal stretch marks. Some reports say oil pack improves the function of the thymus gland, abdominal castor oil pack, improves the production of lymphocytes. It works in fatigue and depression. It can be applied to the skin in various ailments like ring worm, fungal and bacterial infections, wounds, sebaceous cysts, warts, muscle strains, ligament sprains and itching **[8]**.

Various compounds are isolated from leaf of castor plant like flavonoids, glycosides, ricinnine and monoterpenoid and from stem ricinnine and lupeol **[9]**. Castor seed oil has ricinoleic acid which is effective in preventing the growth of numerous species of bacteria, yeast and moulds **[10]**. It shows antidiabetic, wound healing, larvicidal, mollusccidal and antiulcer activity **[11], [12], [13]**.

There are a number of reports available on the antimicrobial activity of different plants which proved to be very effective against variety of pathogenic bacteria **[14], [15].** Antimicrobial property of leaf, stem, seed and root extracts of this plant were studied by **[3], [16], [17].**

3. Problem Definition

In view of these earlier reports present work is undertaken to investigate antimicrobial potential of fresh leaves, stem and tender fruits of *R. communis.*

4. Methodology

Plant Material: *R. communis* was collected in the month of March from pollution free zone of Sanjay Gandhi National Park, Mumbai, separated into individual leaves, stem and tender fruits and cleaned thoroughly. Fresh plant material was used for the preparation of extracts.

Preparation of Extracts: Extracts were prepared using individual fresh plant parts stem, leaves and tender fruits by homogenizing it in mortar and pestle using solvents like acetone and petroleum ether. 10 grams of fresh plant samples were homogenized in each solvent and extraction was allowed for 45 minutes, filtered and final volume was

made to 20 ml by adding required amount of solvent. The extracts were stored in air tight borosil bottles at 4°C.

Microorganisms: Six gram negative bacteria *Escherichia coli (E.col), Klebsiella pneumonia (K.p), Salmonella typhi (S.t), Salmonella paratyphi A (Sp.A), Salmonella paratyphi B (Sp.B)* and *Pseudomonas aeruginosa (Ps.aure),* four gram positive bacteria *Staphylococcus aureus (S.a), Bacillus subtilis (B.subt), Streptococcus pyogens (S.pyo)* and *Corynebacterium diphtheriae (C.d)* were used for bioassay. These were collected from Haffkine Institute, Parel, Mumbai. All the bacteria sub cultured at regular intervals and stored at 4°C.

Bioassay: Primary screening and MIC tests were carried out by Agar Cup method **[18]**. Sterile sugar tubes with 20-25 ml sterile nutrient agar and 0.4 ml of culture made in nutrient broth of each pathogen, mixed and poured on each sterile plate. The plates were kept aside for agar solidification. With diameter 8 mm wells were made using cork borer with 8 mm diameter. Three of these wells were filled with 80µl of crude extracts and fourth one was filled with negative control, a solvent in which extract is made. The results were observed as clear inhibition zone around the wells after 24 hrs.

Minimum Inhibitory Concentration (MIC): In primary screening, the crude extracts which displayed zone of inhibition more than 15 mm in diameter were selected for MIC studies. Each extract was evaporated at room temperature to dry residues and than using fresh solvent, different concentrations viz.10mg/ml, 5mg/ml, 1mg/ml and 0.5mg/ml were prepared. The bioassay was conducted with all concentrations by above mentioned agar cup method.

5. Results

All the extracts of acetone made in fresh stem, leaves and tender fruits showed promising antimicrobial activity than all the extracts of petroleum ether (**Table- I & II**). Range of zone of inhibition was 12 mm to 25 mm against susceptible test microorganisms. Highest zone of inhibition (25 mm) was noted against *K. pneumonia* and *Ps. aeruginosa* by acetone leaf extract and by acetone tender fruit extract against *Ps. aeruginosa*. It was seen that *E. coli, S. typhi, S. paratyphi A* and *S. paratyphi B* were highly resistant against all acetone extracts. Petroleum ether extracts displayed very mild zone of inhibition against *S. pyogens, S. aureus, K. pneumonia* and *C. diphtheriae*. Remaining test pathogens were highly resistant against all petroleum ether extracts.

MIC of potent extracts of acetone showed inhibitory activity at 0.5 mg/ml against susceptible human bacteria (**Table-III**).

6. Discussion:

In this study mid-polar solvent, acetone displayed better bioactivity than non-polar solvent petroleum ether. All acetone extracts showed inhibitory activity against *B. subtillis, S. pyogens, S. aureus, C. diphtheriae, K. pneumonia* and *Ps. aeruginosa*. This indicates that mild to strong active principles can be dissolved in mid-polar solvents. Similar results were found by Sharma , *et. al*., **[9]** which shows less activity in leaf extracts of *R. communis* prepared in petroleum ether. In this work the effective solvent was acetone. Similar observations are recorded by Kensa and Syhed **[16]** with root, stem and leaves extract against *K. pneumonia, E. coli* and *S. aureus*. Other investigation was by Tajamul *et. al.*,**[19]** of leaf extracts against *E. coli, S. aureus, K. pneumonia* and *S. pyogens*.

These findings are in accordance with these reports. Therefore it is suggested that acetone solvent is good for extraction of phytoconstituents from plants.

7. Conclusion

Present study confirms that the bioactive principles of all parts of *R. communis* acetone extracts are highly active against sensitive gram positive and gram negative bacteria. Thus it can be concluded that the extracts of leaves, stem and tender fruits possess substantial amount of strong bioactive broad spectrum phyto-constituents.

8. Future Scope

It is suggested that the entire plant may be further explored for their active ingredients and further investigation is needed for its chemical composition in view of its pharmaceutical importance.

Table 1: Antimicrobial activity of *R. communis* of acetone extract on pathogens. (Inhibition Zones in mm)

	B.subt	*S.pyo*	*S.a*	*K.p*	*C.d*	*E.coli*	*S.t*	*Sp.A*	*Sp.B*	*Ps.aeru*
Leaf	20	-	21	25	19	-	-	-	-	25
Stem	18	-	23	13	19	-	-	-	-	22
Fruit	14	16	-	23	14	-	-	-	-	25

Keywords: No zone of inhibition.

Table 2: Antimicrobial activity of *R. communis* of petroleum ether on pathogens (Inhibition Zones in mm)

	B.subt	*S.pyo*	*S.a*	*K.p*	*C.d*	*E.coli*	*S.t*	*Sp.A*	*Sp.B*	*Ps.aeru*
Leaf	-	12	12	12	14	-	-	-	-	-
Stem	-	14	13	12	-	-	-	-	-	-
Fruit	-	12	12	12	-	-	-	-	-	-

Keywords: No zone of inhibition.

Table 3: MIC of Acetone extract

Plant part	Pathogen	Concentration mg/ml.			
		10	5	1	0.5
Leaves	*B.subt*	20	15	12	-
	S.a	20	17	14	-
	K.p	27	20	16	14
	C.d	20	16	13	-
	Ps.aeru	26	21	18	14
Stem	*B.subt*	18	14	11	-
	S.a	24	21	17	16
	C.d	21	17	13	11
	Ps.aeru	24	20	16	13
Tender fruit	*S.pyo*	16	14	11	-
	K.p	26	19	16	12
	Ps.aeru	27	20	15	12

Keywords: - No zone of inhibition.

Bioassay of acetone extract against *K. pneumonia*

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Author Profile

Prof. (Dr.) Chhaya S. Mitter is Head of Zoology Department, Sathaye College, Vile Parle (E), University of Mumbai, Mumbai-400057, Maharashtra, India. Associate Professor for 26 years. Successfully completed university research projects. Published various research papers. Worked on various bodies in the college and university. Active in social fields. Worked as associate editor for Bio-chemical Science.