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Antimicrobial Activity of *Catunaregam spinosa* (Thumb.) Tirveng -An important medicinal plant

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Abstract

The ethanol, petroleum ether, benzene, chloroform and aqueous extracts of the leaf and stem of ***Catunaregam spinosa***, were screened for their anti-microbial sensitivity against 10 human pathogenic bacteria.

Key words: *Catunaregam spinosa*, Gram⁻ve & Gram ⁺ve bacteria, antimicrobial activity.

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1. Introduction

The WHO estimated that 80% of the people living in developing countries almost exclusively use traditional medicine. Most of the traditional medicine relies heavily on medicinal plants (Eloff, 1998). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko *et al.,* 2002). The experimental material selected for the present study *Catunaregam spinosa* belongs to the family Rubiaceae. It is also known as a *Randia dumetorum*. It is a large deciduous thorny shrub growing up to five meters of height and it occurs almost throughout in India up to 4,000 ft altitude. This root contains triterpene, -1-Quito-3-hydroxyoleanane, this park contains mannitol, saponins, coumarin glycosides, and leaves contain an iridoid-10- methylixoside. An iridoid glycoside and ripe fruit contains glycosides, randioside A, mollisidial triterpenoid glycosides and randianin, six saponins-dumetoronins A to F. Traditionally, this plant used as cure abscess, ulcers, inflammation, wounds, tumors, skin diseases. The pulp of the fruit also has anthelmintic properties, and also used as an abortiffcient as a folklore remedy (Patel *et al.*, 2011). Therefore, in the present study, the extracts of *Catunaregam spinosa* were screened for its antimicrobial sensitivity against 10 human pathogenic bacteria and thus ascertain its Bio-efficiency.

2. Material and Methods

The leaf and stem were air dried and grounded into a coarse powder. For aqueous extraction, 10 g of air-dried powder was mixed with distilled water and boiled on slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected. The same procedure was repeated twice. After 6 h, the supernatant, collected at an interval of every 2 hours, was pooled and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved (121^0C, 15 lbs pressure) and stored at 4^oC. For solvent extraction, 10 g of air dried powder was mixed with 100 ml of organic solvent (methanol or ethanol, chloroform, petroleum ether, benzene) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190 - 220 rpm for 24 hours. After 24 hours, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatants were collected in 2 ml Eppendorf tubes separately and opened for condensation. The filter paper discs were placed in the Eppendorf tubes and impregnated well with the extracts for condensation. The impregnated filter paper discs were used to study the sensitivity assay against pathogens.

Fig.1: *Catunaregam spinosa* (Thumb.) Tirveng

2.1 Preparation of culture media

The petriplates were washed well and dried under hot air oven. Then the petriplates were sterilized at 121^0C, 15 lbs pressure. The Muller Hinton Agar was taken and sterilized at 121^0C for 15 -20 minutes. After sterilization, the media was poured into petriplates (15 -20 ml) under the Laminar Air Flow Hood Chamber and allowed to cool.

2.2 Antimicrobial activity test

Standard bacterial strains were used for screening these were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhii*, *Streptococcus pyogens*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterobacter aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis.* These bacterial species were cultured in broth separately. After 12-18 hours, they were swapped uniformly on petriplates under the Laminar Air Flow Hood chamber. The impregnated filter paper discs were placed on the culture media swapped by different pathogens. Along with that control discs from the solvent (Negative control) and antibiotic disc (positive control) were screened against pathogenic bacteria. The cultures were incubated at 32 ± 2^0C for 18 hours. After 18 hours, the zone of

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inhibition were measured by a graduated scale and tabulated. The activity was measured by zone of inhibition in millimeter.

Table-1: Effect of *Catunaregam spinosa* leaf extract against human pathogenic bacteria

S. No	Name of the pathogens	Strains type	Zone of inhibition of *Catunaregam spinosa* leaf extracts (mm)						
			Ethanol	Chloroform	Benzene	Petroleum Ether	Dis.H_2O	*positive control*	*Negative control*
1	*Escherichia coli*	G^{-ve}	10	-	-	-	-	14	-
2	*Pseudomonas aeruginosa*	G^{-ve}	10	-	-	-	-	23	-
3	*Staphylococcus aureus*	G^{+ve}	8	7	-	-	-	18	-
4	*Salmonella typhii*	G^{-ve}	13	10	-	-	-	22	-
5	*Streptococcus pyogens*	G^{+ve}	10	-	-	-	-	20	-
6	*Serratia marcescens*	G^{-ve}	-	7	7	-	-	22	-
7	*Klebsiella pneumoniae*	G^{-ve}	7	-	-	-	-	15	-
8	*Enterobacter aeruginosa*	G^{-ve}	14	10	-	-	-	18	-
9	*Proteus vulgaris*	G^{-ve}	9	-	9		-	21	-
10	*Bacillus subtilis*	G^{+ve}	8	8	*10*	-	-	18	-

- Negative result

Table-2: Effect of *Catunaregam spinosa* stem extract against human pathogenic bacteria

S.No	Name of the pathogens	Strains type	Zone of inhibition of *Catunaregam spinosa* stem extracts (mm)						
			Ethanol	Chloroform	Benzene	Petroleum ether	Dis.H	*positive control*	*Negative control*
1	*Escherichia coli*	G^{-ve}	10	13	8	-	-	15	-
2	*Pseudomonas aeruginosa*	G^{-ve}	12	-	-	-	-	30	-
3	*Staphylococcus aureus*	G^{+ve}	24	20	17	12	-	22	-
4	*Salmonella typhii*	G^{-ve}	10	8	-	-	-	21	-
5	*Streptococcus pyogens*	G^{+ve}	13	-	-	-	-	17	-
6	*Serratia marcescens*	G^{-ve}	12	7	-	-	-	24	-
7	*Klebsiella pneumoniae*	G^{-ve}	25	27	25	15	16	25	-
8	*Enterobacter aeruginosa*	G^{-ve}	8	8	7	-	-	24	-
9	*Proteus vulgaris*	G^{-ve}	16	10	-	-	-	27	-
10	*Bacillus subtilis*	G^{+ve}	18	-	17	-	-	20	-

- Negative result

3. Result and Discussion

The leaf extracts of ethanol, chloroform and benzene were effective against all the pathogens (Plate-1). Ethanolic extract of the leaf showed the maximum of 14 mm in diameter was observed against *Enterobacter aeruginosa* and 13 mm in diameter against *Salmonella typhii*. The minimum of 7 mm in diameter was observed against 3 pathogenic bacteria namely *Staphylococcus aureus, Serratia marcescens and Klebsiella pneumoniae.* Chloroform extract of the leaf showed antibacterial activity against 5 pathogenic bacteria. It had maximum inhibitory action against *Salmonella typhii* (10 mm) and *Enterobacter aeruginosa* (10 mm). Benzene extract of the leaf showed antibacterial activity against three pathogenic bacteria namely *Serratia marcescens* (7mm), *Proteus vulgaris* (9 mm) and *Bacillus subtilis* (10mm). Petroleum ether and aqueous extract of the leaf had no inhibitory action against the pathogenic bacteria (Table-1 and 2)

The stem extract was effective against all the pathogenic bacteria especially. *Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis.* In carvacrol, together with thymol,

inhibits *Bacillus subtilis, Salmonella enteritidis, S.aureus, Pseudomonas aeruginosa, Pseudomonas morganii* and *Escherichia coli* **(**Conner, 1993). Catechol is a hydroxylated phenol which is toxic to microorganisms (Cowan, 1999). An Ethanolic extract of the leaf showed the maximum of 25 mm in diameter was observed against *Klebsiella pneumoniae* and the minimum of 8 mm in diameter was observed against *Enterobacter aeruginosa* Chloroform extract of the stem showed maximum antibacterial activity against *Klebsiella pneumoniae* (27 mm) and it had minimum inhibitory action against *Serratia marcescens* (7 mm). Benzene extract of the stem showed antibacterial activity against 5 pathogenic bacteria. It was highly effective to control the growth of *Klebsiella pneumoniae* (25 mm). Petroleum ether and aqueous extract of the stem had inhibitory action against only three pathogenic bacteria (Table - 2). The activity was measured by zone of inhibition in mm (Dhavan *et al*., 1980). The positive results were compared with that of the reference antibiotic (Gentamycin) and it was found that many extracts in equal proportion with the antibiotic against the pathogenic bacteria. The stem extract was highly effective to control the growth of all the pathogenic bacteria than the leaf extract.

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