



Research Article

ANTI BACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *CLERODENDRUM INEREME*

Arvindganth Rajasekar*, Hemalatha S

Department of Biotechnology, VELS University, Pallavaram Chennai, India

*Corresponding Author Email: santhiarvind.007@gmail.com

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ABSTRACT

The aim of the present study is to investigate the antimicrobial activity, anticancer and phytochemical analysis of different extracts in *Clerodendrum inereme*. The solvents chloroform and hexane were used to extract the bioactive compounds from the leaves for their antimicrobial activity against different strains of bacteria by disc diffusion method. The maximum antibacterial activity was observed in crude chloroform extract of *Clerodendrum inereme* and analysis of phytochemical screening reveals the presence of Alkaloids, Saponins, Tannins, Steroids, Glycosides and Flavanoids.

Keywords: *Clerodendrum inereme*, antibacterial activity and phytochemical analysis.

INTRODUCTION

Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are deterrent. As prevent you and curative measures plants and their products are used in the treatment of infections for many centuries ago. Who estimated that 80 % of the people worldwide rely on plant based medicines for their primary healthcare¹ and India happens to be the largest user of traditional medical cure, using 7000 plant species. The increase in failure of chemotherapies and antibiotic resistances exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity^{2,3}. Antibacterial properties of various plants, such as leaves, seeds and fruits have been well documented for some of the medicinal plants for the past two decades⁴. Antibiotic principles are distributed widely among angiospermic plant. A variety of are compounds are accumulated in plants part accounting for their constitutive antimicrobial activities⁵. Medicinal and aromatic plants are potential source of raw materials used for manufacture of drugs and perfumery products more than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment⁶.

MATERIALS AND METHODS

Collection and Identification of Plant

The *Clerodendrum inereme* plant, leaves was collected from Vaniyambadi, Vellore district, Tamil Nadu, India. The plant was confirmed by Dr. Kathiravan Ph. D (Assition professor in Department of Biotechnology in Vels University, Pallavaram Chennai, India. Herbarium number VUBT1000. (Vels University)

Preparation of Plant Extracts

The fresh plant *Clerodendrum inereme* sample (leaves) were collected and washed under the running tap water to remove soil particles and other dust particles. The leaves were air dried under the laboratory condition at room temperature for 15 days. The dried leaves samples were ground well in to a fine powder with the help of mixer grinder. A 10 g air dry plant powder *Clerodendrum inereme* was soaked into 50 ml organic solvents, viz, Methanol, Chloroform and Hexane separately for 24 h in a orbital shaker at normal temperature. The extracts were filter through the Whatman No: 1 filter paper. The extract was allowed to dry using rotary evaporator. The condensed extracts were stored in airtight container at 4°C till further investigation.

Microbial Cultures

Pathogenic microbial cultures of *Escherichia coli* (MTCC553), *Bacillus subtilis* (MTCC121), *Pseudomonas aeruginosa* (MTCC890), *Streptococcus mutans*, *micrococcus*, *Klebsiella pneumoniae*, *Salamonella typhi* were obtained from the University of Madras, Chennai, India.

Solvents used

Chloroform and Hexane were used as the solvents for the preparations of *Clerodendrum inerme* extracts.

Phytochemical analysis

Phytochemical screening was performed to identify phytochemicals in the chloroform and hexane extracts of the *Clerodendrum inerme* (leaves) used in the study in this present work, the phytochemicals were detected by color tests.

Test for Alkaloids

Of each extract 2 ml was acidified with a few drops of dilute hydrochloric acid. Then 1 ml of dragendroff reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

Test for Tannins

To 2 ml of each extract a few drops of 10 % lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

Test for Saponins

To 1 ml of extract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 seconds and extract were allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

Test for Steroids

Chloroform 10 ml was added to 2 ml of all three plant extracts. To these extracts 1ml of acetic anhydride was added and then 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Color formation at the junction is noted. The appearance of blue green color indicates the presence of steroids.

Test for Triterpenoids

The test for triterpenoids is same as that for steroids the appearance of red, pink color or violet color at the junction indicates the presence of triterpenoids.

Test for Glycosides

To 1 ml of each extract a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentration sulphuric acid were added. The appearance of blue-green color indicates the presence of glycosides.

Test for Flavonoids

4 ml of extract solution was treated with 1.5 ml of methanol solution. The solution was warmed and metal magnesium was added to this solution 5-6 drops of con. HCl acid was added and color was observed for flavonoids and orange color for flavones.

Test for Reducing Sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehlings solution was added to the test tube hot and observed for brick red precipitate.

**Antimicrobial Activity
Disc Diffusion Method**

Antimicrobial activity was evaluated using the agar diffusion technique in petri dishes, 25 ul, 50 ul, 75 ul of *Clerodendrum inerme* extracts were loaded on sterile filter paper discs 5 mm in diameter and air dried. 24 h bacterial cultures were spread on Nutrient Agar plates under sterile condition in laminar air flow and the discs were placed on plates. After incubation for 24 h at temperature, a clear zone around the disc was evidence of anti microbial activity. Diameters of the zone of inhibition were measured in millimeters. Each test was prepared in duplicate. Disc loaded with extracting agents were tested as controls.⁷

RESULTS**Phytochemical Analysis**

The phytochemical test was done by *Clerodendrum inerme* extracts with two different solvents, chloroform and hexane were done by color test. The results were presented in Table 1.

Table 1: Phytochemical present in the chloroform and hexane extract of *Clerodendrum inerme*

Phytochemical Testes	Chloroform	Hexane
Alkaloids	+	-
Tannins	+	-
Saponins	+	-
Steroids	+	+
Triterpenoids	-	-
Glycosides	+	-
Flavonoids	+	+
Reducing Sugars	-	-

(+) Present, (-) Absent

Antimicrobial Activity

Antimicrobial activity of the *Clerodendrum inerme* extracts at 25 ul, 50 ul, 75 ul were tested against the bacterial strain such as *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginose*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Micrococcus sp.*, *Salmonella typhi*. The antimicrobial activity of the *Clerodendrum inerme* chloroform and hexane extract showed inhibitory activity against all the test bacterial strains (plate no: 1) and their zone of inhibition were measured (Table 2-3)

Table 2: Antimicrobial Activity of Chloroform Extract of *Clerodendrum inerme*

S. No.	Organism	Concentration (mm)	25 ul	50 ul	75 ul
1	<i>Escherichia coli</i>	Test	6 mm	10 mm	12 mm
		Ampicillin	11 mm		
		Solvent	3 mm		
2	<i>Bacillus subtilis</i>	Test	5 mm	7 mm	9 mm
		Ampicillin	10 mm		
		Solvent	2 mm		
3	<i>Pseudomonas aeruginosa</i>	Test	2 mm	3 mm	5 mm
		Ampicillin	10 mm		
		Solvent	2 mm		
4	<i>Streptococcus mutans</i>	Test	8 mm	12 mm	14 mm
		Ampicillin	9 mm		
		Solvent	2 mm		
5	<i>Klebsiella pneumoniae</i>	Test	6 mm	8 mm	15 mm
		Ampicillin	10 mm		
		Solvent	2 mm		
6	<i>Micrococcus sp.</i>	Test	5 mm	8 mm	9 mm
		Ampicillin	6 mm		
		Solvent	1 mm		
7	<i>Salmonella typhi</i>	Test	9 mm	12 mm	15 mm
		Ampicillin	9 mm		
		Solvent	1 mm		

Table 3: Antimicrobial Activity of Hexane Extract of *Clerodendrum inerme*

S. No.	Organism	Concentration (mm)	25 ul	50 ul	75 ul
1	<i>Escherichia coli</i>	Test	7 mm	10 mm	12 mm
		Ampicillin	9 mm		
		Solvent	4 mm		
2	<i>Bacillus subtilis</i>	Test	2 mm	4 mm	5 mm
		Ampicillin	9 mm		
		Solvent	nil		
3	<i>Pseudomonas aeruginosa</i>	Test	5 mm	12 mm	14 mm
		Ampicillin	10 mm		
		Solvent	nil		
4	<i>Streptococcus mutans</i>	Test	7 mm	10 mm	13 mm
		Ampicillin	9 mm		
		Solvent	1 mm		
5	<i>Klebsiella pneumoniae</i>	Test	5 mm	9 mm	13 mm
		Ampicillin	9 mm		
		Solvent	nil		
6	<i>Micrococcus sp.</i>	Test	6 mm	8 mm	9 mm
		Ampicillin	7 mm		
		Solvent	nil		
7	<i>Salmonella typhi</i>	Test	8 mm	10 mm	12 mm
		Ampicillin	8 mm		
		Solvent	nil		

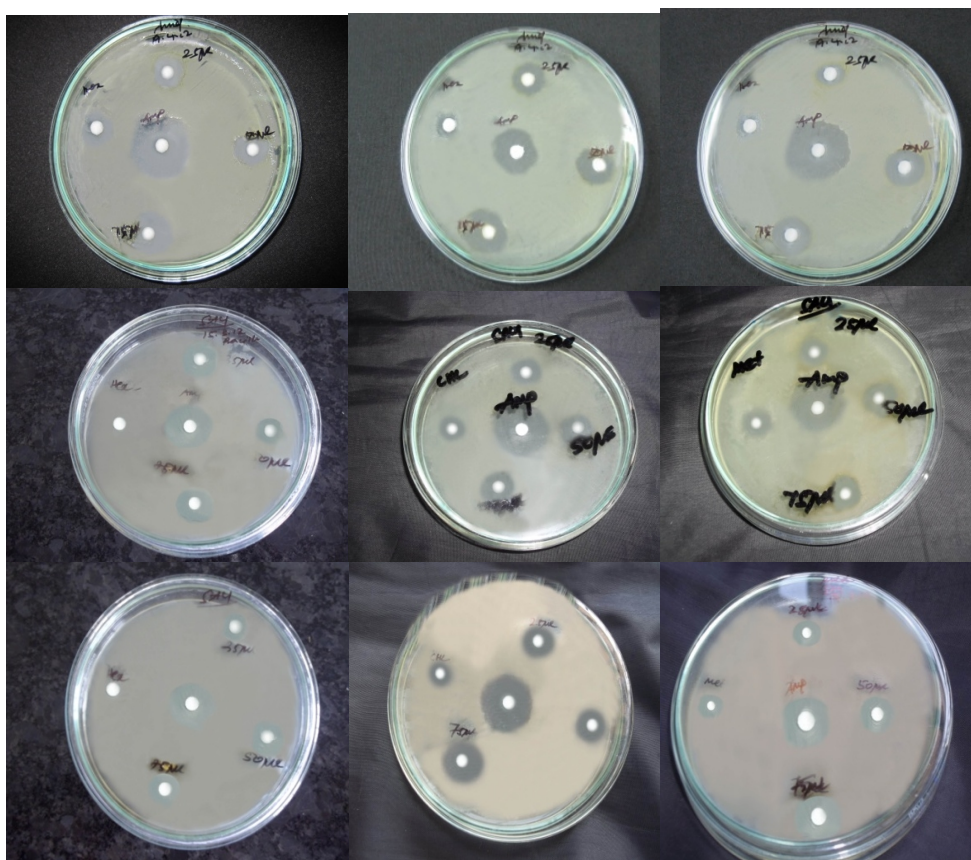


Plate 1: Zone of inhibition against Bacterial strains

DISCUSSION

In the present study of the antimicrobial efficacy of various solvent extracts namely chloroform and hexane of the *Clerodendrum inerme* plants against the pathogenic bacteria showed varied level of inhibition. The activity of the different extracts of *Clerodendrum inerme* were compared with standard drugs ampicillin and solvent served as a control. All the solvent extracts of *Clerodendrum inerme* showed very good activity against the test bacteria. The chloroform extract of *Clerodendrum inerme* have shown maximum zone of inhibition such as 15 mm, 14 mm and 12 mm against *Salmonella*

typhi, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Escherichia coli*, at 75 µg; whereas minimum zone of inhibition was observed in *Pseudomonas aeruginosa* (5 mm). The hexane extract of *Clerodendrum inerme* have shown maximum zone of inhibition such as 14 mm, 13 mm and 12 mm against *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Escherichia coli*, at 75 µg; whereas minimum zone of inhibition was observed in *Bacillus subtilis* (5 mm). Their fore their inhibition active indicate that *Clerodendrum* have potential antimicrobial activity.

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