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PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF N-HEXANE LEAF EXTRACT OF *CLERODENDRUM SERRATUM* LINN AGAINST PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

Clerodendrum serratum Linn.is widely distributed plant in India and its medicinal use has been mentioned in traditional Indian medicine systems. In the present study phtochemical investigatin and evaluation os antibacterial activity usinf disc diffusion method of n-Hexane leaf extract *Clerodendrum serratum* Linn was carried out against pathogenic bacteril strains viz., *staphylococcus hominis* ATCC27844, *Pseudomonas putida* ATCC2021, *Proteus vulgaris* ATCC13315, *Bacillus subtilis* ATCC2063 and *Escherichia coli* ATCC2065. Among the various concentrations used, 100mg/ml of n-Hexane leaf extract was found to be more effective against all tested microorganisms. The highest activity was recorded aganist *Bacillus subtilis* ATCC2063 at a concentration of 100mg/ml with zone of inhibition 20±0.25mm and the lowest activity was recorded against *Pseudomonas putida* ATCC2021 with zone of inhibition17.1±0.40mm. At a concentration of 25mg/ml, the maximum inhibitory activity was recorded against *Escherichia coli* ATCC2065 with zone of inhibition 3.62±0.51mm and the minimum inhibitory activity was recorded against *Pseudomonas putida* ATCC2021 with zone of inhibition 2.5±0.30mm. Therefore, it was found that the inhibitory activity of n-Hexane leaf extract of *Clerodendrum serratum* Linn. was concentration dependent.

KEYWORDS: Clerodendrum Serratum Linn, Staphylococcus Hominis, Pseudomonas Putida, Proteus Vulgaris, Bacillus Subtilis and Escherichia Coli.

INTRODUCTION

Plant kingdom represents an enormous reservoir of biologically active compounds and so far only a fraction of these plants have been assayed. Nearly 50% of the drugs used in medicine are of plant origin. It is important to use phytochemical methods to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. The plants that possess therapeutic properties exert beneficial pharmacological effects and are generally designated as "Medicinal plants". Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile, convulsion, diarrhea, and dysentery, fungal and bacterial infections.[1]

Many plants are found to contain chemical compounds, which are used as natural medicines to treat common bacterial infections. These medicinal plants have been regularly used in various system of Indian medicine because of minimal side effect and cost effec-tiveness which provide scientific support to the therapeutic use of the plants in tribal medicine.[2] The genus *Clerodendrum* L. is widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. Many species of the genus have also been documented in traditional systems of medicine practiced in countries like India, China, Korea, Thailand and Japan.[3] A number of species from the genus *Clerodendrum* are documented in ancient texts for their antimicrobial action.. Roots and leaf extracts of *Clerodendrum* have been used for the treatment of rheumatism, asthma and other inflammatory diseases[4-8] and also possess sedative, antihypertensive and antidiabetic properties.[9-10] The present investigation is conducted for screening anti-bacterial compounds from natural resources as the existing drugs are getting less effective due to increased tolerance of microorganisms. The use of plants to heal diseases, including infectious one, has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds. Once extracted, and before being used in new therapeutic treatments, they should have their toxicity tested. The *in vivo* bioassays.[11-12] have demonstrated the toxicity of extracts from different plants. Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

Clerodendrum Serratum LINN. belongs to family Verbenaceae is a small perennial shrub growing in moist deciduous forests and occasionally in plains of peninsular India and the Western and Eastern Himalayas up to 1,400 feet above sea level. The leaf and root of this plant have great medicinal value. Ethnopharmacological and ethnobotanical knowledge are percolating down to these days among the tribal population, but much of this information is empirical at best, and lacks preclinical scientific validations Therefore, the present study has been taken to validate the traditional claims associated with this plant and to carryout phytochemical investigation and evaluation of antibacterial activity of methanolic extract of *Clerodendrum Serratum LINN.*

MATERIAL AND METHODS

Plant material collection and identification

The healthy leaves of *Clerodendrum searratum* LINN. were collected from Ekant forest park, Bhopal, India. The plant was identified and authenticated by Dr. Ziaul Hassan, Professor of Botany, Saifia Science College, Bhopal, India. A voucher specimen No.305/Bot/Saifia/11 has been submitted to the Department of Botany of Saifia Science College, Bhopal, India for further reference.

Preparation of plant material

The collected leaves of *Clerodendrum searratum* LINN. Were thoroughly washed in running tape water and then shade dried. The completely shade dried leaves were homogenised to coarse powder and stored in air tight containers till further use.

Extraction process

A quantity of 100gm of powdered leaves of *Clerodendrum searratum* LINN. was extracted successively by Soxhelet apparatus with 500 ml of methanol (solvent) for a span of 72 hours. The temperature of methanol was kept at 80±5ºc.The extract was filtered using Whatman's No.1 filter paper. The filtered extract was evaporated and concentrated in water bath at a temperature of 40ºc.The extract was preserved in air tight container till further use.

Test Microorganisms

The anti-bacterialactivity of aqueous leaf extract of *Clerodendrum searratum* Linn. was tested individually against gram-positive and gram-negative bacterial strains. The gram-positive bacterial strains used were *Proteus vulgaris* ATCC13315, *Staphylococcus hominis* ATCC27844 and *Bacillus subtilis* ATCC2063. The gram-negative bacteria used were

Escherichia coli ATCC2065 and *Pseudomonas putida* ATCC2021. All bacterial strains were procured in lyophilized form from Gandhi Medical College, Bhopal, India. All the bacterial strains were maintained at 4°c in nutrient agar medium as bacterial slants.

Anti-bacterial assay

The antibacterialactivity of ethyiacetate leaf extract of *Clerodendrum searratum* LINN. was assessed by using disc diffusion method.[13] For inoculum preparation, Mueller- Hinton broth media, qualigens fine chemicals, India was prepared at a concentration of 38 gms/1000 ml of distilled water. The prepared medium was sterilized by autoclaving at a temperature of 121˚c for 15 minutes at 15psi.Under aseptic conditions in laminar airflow cabinet, bacterial strains were transferred into 5ml of Mueller- Hinton broth media using inoculation loop to obtain a bacterial suspension having density of 10 CFU /ml. After this, a quantity of 15ml of Mueller-Hinton agar was poured into each Petri plate to yield a uniform depth of 3mm and then it was then allowed to solidify. After solidification, inoculum of 20ml was dispensed into each Petri plate and thoroughly spreaded using spreader and this technique is known as spread plate technique. Whatman's No.1 Filter Paper was cut into small discs of 6mm diameter and were autoclaved. The autoclaved discs were then dipped into four different concentrations namely 25mg/ml, 50mg/ml, 75mg/ml & 100mg/ml of ethyiacetate leaf extract of *Clerodendrum serratum* LINN. The saturated discs were placed on the inoculated surface and incubated at a temperature of 37˚c for 24 hours. The drug Tetracycline was used as a standard and was available in the concentration of 10 µg /ml. The water was used as a negative control. The result of anti-bacterialactivity was obtained by measuring the diameter of the zone of inhibition. The experiment was performed under strict aseptic conditions for three times to minimize error and the mean values are presented in Table 2.

Statistical Analysis

The resultant clear zones around the discs were measured in mm. The anti-bacterialactivity of leaf extracts was indicated by clear zone of growth inhibition. The values obtained are mean inhibition zone (mm) ± standard deviation of three replicates.

RESULTS AND DISCUSSION

The results of the antibacterial activity of n-Hexane extract leaf extract of *Clerodendrum serratum* Linn. against pathogenic bacterial strains were presented in Table 2. At a concentration of 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of n-Hexane extract leaf extract against *Escherichia coli* ATCC2065, the zones of inhibition recorded were 3.62±0.51 mm,

5.87±0.49mm, 8.2±0.43mm and 18.4±0.45 mm. Here, the maximum antibacterial activity was recorded at a concentration of 100mg/ml with the zone of inhibition 18.4±0.45 mm and the minimum antibacterial activity was recorded at a concentration of 25 mg/ml with the zone of inhibition of 3.62±0.51 mm. The discs impregnated with 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of 3.62±0.51 mm 3.62±0.51 mm of n-Hexane extract of leaf extract of *Clerodendrum serratum* Linn. against *Bacillus subtilis* ATCC2063, the zones of inhibition recorded were 4±0.22 mm, 6.3±0.71mm, 9.7±0.45 mm and 20±0.25 mm. Here, the maximum antibacterial activity was found at a concentration of 100mg/ml with the zone of inhibition 20±0.25 mm and the minimum antibacterial activity was recorded at a concentration of 25 mg/ml with zone of inhibition of 4±0.22 mm. The discs impregnated with 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of n-Hexane leaf extract of *Clerodendrum serratum* Linn. against *proteus vulgaris* ATCC13315, the zones of inhibition recorded were 2.6±0.57 mm, 5.9±0.34mm, 9.3±0.59 mm and 19±0.01 mm. Here, the maximum antibacterial activity was found at a concentration of 100mg/ml with the zone of inhibition of 19±0.01 mm and the minimum antibacterial susceptibility was recorded at a concentration of 25 mg/ml with zone of inhibition of 2.6±0.57 mm. For *Pseudomonas putida* ATCC2021, the concentration of n-Hexane leaf extract used were 25mg/ml,50mg/ml,75mg/ml & 100mg/ml and the zones of inhibition recorded were 2.5±0.30 mm,3.32±0.74 mm,7.24±0.99 mm and 17.1±0.40 mm respectively. The highest antibacterial activity was observed at a concentration of 100mg/ml with zone of inhibition of 17.1±0.40 mm and the lowest activity was recorded at a concentration of 25mg/ml with zone of inhibition of 2.5±0.30 mm. The discs impregnated with 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of n-Hexane leaf extract of *Clerodendrum serratum* Linn. against *staphylococcus hominis* ATCC27844, the zones of inhibition recorded were 3.1±0.25 mm, 5.7±0.75mm, 8.1±0.71mm and 18.2±0.27mm. Here, the maximum susceptibility was found at a concentration of 100mg/ml of leaf extract with the zone of inhibition of 18.2±0.27mm and the minimum antibacterial activity was recorded at a concentration of 25 mg/ml with zone of inhibition of 3.1±0.63 mm. Among all the tested microorganism, *Bacillus subtilis* ATCC2063, showed highest antibacterial activity with zones of inhibition ranging from 4±0.22mm to 20±0.25mm and *Pseudomonas putida* ATCC2021 was found to be least susceptible with zones of inhibition ranging from 2.5±0.30mm to 17.1±0.40 mm. The standard drug tetracycline at a concentration of 10 µg/ml exhibited strong antibacterial activity with zones of inhibition ranging from 18±0.47mm to 24±0.20mm against all tested micro-organisms. Moreover, the n-Hexane leaf extract was subjected to minimum inhibitory concentration (MIC) by employing disc diffusion method. The results of

the MIC are shown in Table 3.The MIC of n-Hexane extract against *Escherichia coli* ATCC2065, *Bacillus subtilis* ATCC2063,*Proteus vulgaris* ATCC13315, *Pseudomonas putida* ATCC2021 and *Staphylococcus hominis* ATCC27844 were found to be 18.92mg/ml,18.98mg/ml,19.88mg/ml,20.11mg/ml and 19.5 mg/ml respectively. It was also observed that with an increase in concentration of aqueous leaf extract, there was an increase in the antibacterial activity against tested pathogenic bacterial strains. It indicates that the antibacterial activity is concentration dependent.

Table. 1: Phytochemical analysis of *aqueous leaf extract of Clerodendrum Serratum* Linn.

Test	Results
Carbohydrates	+ve
Alkaloids	+ve
Glycosides	+ve
Phenolics	+ve
Proteins	+ve
Flavonoids	+ve
Carbonate	+ve
Saponin	+ve
Steroids	+ve
Starch	+ve

Table. 2: Antibacterial activity of n-Hexane extract of *Clerodendrum serratum* Linn.

Microbial Strain	Concentration of n-Hexane Extract				
	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Standard
Escherichia coli	3.62±0.51**	5.87±0.49**	8.2±0.43**	18.4±0.45^{ns}	18±0.47
Bacillus subtilis	4±0.22**	6.3±0.71**	9.7±0.45**	20±0.25**	30±0.28
Proteus vulgaris	2.6±0.57**	5.9±0.34**	9.3±0.59**	19±0.01**	25±0.23
Pseudomonas putida	2.5±0.30**	3.32±0.74**	7.24±0.99**	17.1±0.40^{ns}	16±0.21
Staphylococcus hominis	3.1±0.63**	5.7±0.75**	8.1±0.71**	18.2±0.27**	24±0.20

Table. 3: MIC value of n-Hexane leaf extract of *Clerodendrum serratum* Linn.

Microbial Strain	MIC Value (mg/ml)
Escherichia coli	18.92
Bacillus subtilis	18.98
Proteus vulgaris	19.88
Pseudomonas putida	20.11
Staphylococcus hominis	19.50

In general, the antibacterial activity of plant extracts appears to be more inhibitory to Gram-positive bacteria than Gram-negative bacteria. It should be remembered that penicillin and some of the other prominent antibiotic agents of fungal origin are also rather selective in their

inhibitory action, most of them being inhibitory to Gram-positive bacteria. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria.[14] The outer lipopolysaccharide layer hinders access of most compounds to the peptidoglycan layer of the cell wall. This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity. The negative results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria.[15] The antimicrobial extracts of tested plants can be assumed to be useful to. the producing plant in warding off infectious diseases and there is therefore a compelling reason to suppose that anti-infective agents could be active against human pathogens as was suggested by folkloric and historical accounts.[16]

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