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Research Article

ANTIBACTERIAL ACTIVITY OF ETHYLACETATE EXTRACT OF LEAVES OF *CLERODENDRUM SERRATUM* LINN. AGAINST PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

The success of chemotherapy lies in the continuous search for new drugs to counter the challange posed by resistant bacterial strains. The aqueous leaf extract of *Clerodendrum serratum* Linn. tradionally used in folklore medicine for the treatment of various ailments was investigated for in-vitro antibacterial activity aganist five bacterial pathogens namely *staphylococcus hominis* ATCC27844, *Pseudomonas putida* ATCC2021, *Proteus vulgaris* ATCC13315, *Bacillus subtilis* ATCC2063 and *Escherichia coli* ATCC2065.The antibacterial activity of the aqueous extract was determined by employing agar disc diffusion method. Among the various concentrations used, 100mg/ml of aqueous leaf extract was found to be more effective against all tested microorganisms. The highest activity was recorded aganist *Escherichia coli* ATCC2065 at a concentration of 100mg/ml *Proteus vulgaris* ATCC2021 with zone of inhibition 17±0.52mm and the lowest activity was recorded aganist *Proteus vulgaris* ATCC2021 at a concentration of 25mg/ml with zone of inhibition1±0.09mm. Therefore, it was found the inhibitory activity of ethylacetate leaf extract of *Clerodendrum serratum* Linn. was found to be concentration dependent. The results of this study provides justification for the use of this plant in folkloric medicine for the treatment of various dreadful ailments.

INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Since antiquity, many plants species reported to have pharmacological properties as they are known to posses various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, tirpenes which is therefore, should be utilized to combat the disease causing pathogens (Dubey *et.al.,* 2011).

With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections (Lalitha, *et.al*., 2009). However, only one third of the infectious diseases known have been treated from these synthetic products (Sharma, 2011). This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens (Ahmad and Beg, 2001). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Warrier *et.al*., 1995).

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,

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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-bacterial activity 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 antibacterial activity of ethyiacetate leaf extract of *Clerodendrum searratum* LINN. was assessed by using disc diffusion method (Marjorie *et. al*., 1999). 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-bacterial activity 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-bacterial activity of leaf extracts was indicated by clear zone of growth inhibition. The values obtained are mean inhibition zone(mm) ± standard deviation of three replicates.

Ethyl acetate Extract

The results of the antibacterial activity of ethyl acetate leaf extract of *Clerodendrum serratum* Linn. against pathogenic bacterial strains were presented in Table 1. At a concentration of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml of ethyl acetate leaf extract against *Escherichia coli* ATCC2065, the zones of inhibition recorded were 3.35±0.44 mm, 5.2±0.30mm, 7.75±0.60 mm and 17±0.52 mm. Here, the maximum anti-bacterial activity was recorded at a concentration of 100 mg/ml with the zone of inhibition 17±0.52 mm and the minimum anti-bacterial activity was recorded at a concentration of 25 mg/ml with the zone of inhibition of 3.35±0.44 mm.

The discs impregnated with 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml of ethyl acetate leaf extract of *Clerodendrum serratum* Linn. against *Bacillus subtilis* ATCC2063, the zones of inhibition recorded were 1.25±0.55 mm, 2.25±0.72 mm, 3.25±0.59 mm and 10.37±0.39 mm. Here, the maximum activity was found at a concentration of 100 mg/ml with the zone of inhibition 210.37±0.39 mm and the minimum anti-bacterial activity was recorded at a concentration of 25 mg/ml with zone of inhibition of 1.25±0.55 mm. The discs impregnated with 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml of aqueous leaf extract of *Clerodendrum serratum* Linn. against *proteus vulgaris* ATCC13315, the zones of inhibition recorded were 1±0.09 mm, 3.37±0.08 mm, 3±0.05 mm and 5.87±0.12 mm. Here, the maximum activity was found at a concentration of 100 mg/ml with the zone of inhibition 5.87±0.12 mm and the minimum anti-bacterial activity was recorded at a concentration of 25 mg/ml with zone of inhibition of 1±0.09 mm. For *Pseudomonas putida* ATCC2021, the concentration of ethyl acetate leaf extract used were 25 mg/ml, 50 mg/ml,75 mg/ml & 100 mg/ml and the zones of inhibition recorded were 3±0.48 mm, 6.37±0.22 mm, 8.62±0.71 mm and 11.12±0.45 mm respectively. The highest anti-bacterial activity was observed at a concentration of 100 mg/ml with zone of inhibition of 11.12±0.45 mm and the lowest activity was observed at a concentration of 25mg/ml with zone of inhibition of 3.37±0.48 mm. The discs impregnated with 25 mg/ml, 50

mg/ml, 75 mg/ml and 100 mg/ml of ethyl acetate leaf extract of *Clerodendrum serratum* Linn. against *staphylococcus hominis* ATCC27844, the zones of inhibition recorded were 2.31±0.25 mm, 5.1±0.62mm, 7.25±0.56 mm and 10.2±0.40 mm. Here, the maximum activity was found at a concentration of 100 mg/ml of leaf extract with the zone of inhibition 10.2±0.40 mm and the minimum anti-bacterial activity was recorded at a concentration of 25 mg/ml with zone of inhibition of 2.31±0.25 mm. Among all the tested microorganism, *Pseudomonas putida* ATCC2021 showed highest anti-bacterial activity with zones of inhibition ranging from 3.37±0.48 mm to 11.12±0.45 mm and *proteus vulgaris* ATCC13315 was found to be least susceptible with zones of inhibition ranging from 1±0.09 mm to 5.87±0.12 mm. The standard drug tetracycline at a concentration of 10 µg/ml exhibited strong anti-bacterial activity with zones of inhibition ranging from 18±0.47 mm to 24±0.20 mm against all tested micro-organisms. Moreover, the ethyl acetate leaf extract was subjected to minimum inhibitory concentration (MIC) by employing disc diffusion method. The results of the MIC are shown in Table 3. It was also observed that with an increase in concentration of aqueous leaf extract, there was an increase in the anti-bacterial activity against tested pathogenic bacterial strains. It indicates that the anti-bacterial 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 Anti-bacterial activity of ethyl acetate extract of *Clerodendrum serratum* Linn

Microbial Strain	Concentration of Ethyl acetate Extract				
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml	Standard
Esherichia coli	3.35±0.44**	5.2±0.30**	7.75±0.60**	17±0.52^{ns}	18±0.47
Bacillus subtilis	1.25±0.55**	2.5±0.72**	3.25±0.59**	10.37±0.39**	30±0.28
Proteus vulgaris	1±0.09**	3.37±0.08**	3±0.05**	5.87±0.12**	25±0.23
Pseudomonas putida	3.37±0.48**	6.37±0.22**	8.62±0.71**	11.12±0.45**	16±0.21
Staphylococcus hominis	2.31±0.25**	5.1±0.62**	7.25±0.56**	10.2±0.40**	24±0.20

Values are mean inhibition zone (mm) ± S.D of 3 replicates

Table 3 MIC value of ethyl acetate leaf extract of *Clerodendrum serratum* Linn

Microbial Strain	MIC Value (mg/ml)
Esherichia coli	16.11
Bacillus subtilis	18.66
Proteus vulgaris	22.55
Pseudomonas putida	15.55
Staphylococcus hominis	7.22

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 (Vidya *et.al*., 2009). It was observed that the leaf extract exhibited maximum antibacterial activity against *Proteous vulgaris* ATCC13315 at all concentrations with zones of inhibition ranging from 3.35±0.44mm to 17±0.52mm.The minimum antibacterial susceptibility was shown by *Proteous vulgaris* ATCC13315 with zones of inhibition ranging from 1±0.09mm to 5.87±0.12mm. The least antibacterial susceptibility was shown by gram negative bacteria which may be attributed to the presence of an outer lipid layer which impedes the penetration of antibacterial compounds which and thus resulted in less antibacterial activity (Minh, *et.al*., 2009).It is well understood from the results that the maximum antibacterial susceptibility was shown by *Escherichia coli* ATCC2065 at a concentration of 100mg/ml with zone of inhibition of 17±0.52mm.The least antibacterial susceptibility among the tested micro-organisms was exhibited by *Proteous vulgaris* ATCC13315 at a concentration of 25mg/ml with zone of inhibition of 1±0.09mm.This disparity in antibacterial susceptibility of bacterial strains may not be unconnected with the strain variations and difference in mechanism of actions of active molecules present in the aqueous leaf extract. On preliminary phytochemical screening the aqueous leaf extract of *Clerodendrum serratum* Linn. Showed presence of diverse phytoconstituents. Among the present phytoconstituents flavonoids and steroids are responsible for anti-microbial activity of the ethylacetate leaf extract. Flavonoids are hydroxilated phenolic compounds known to be synthesised by plants in response to microbial infection and it acts probably due to ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjori, 1999) .Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compounds indicates the mechanism in which steroids specifically associate with membrane lipids and exerts its action by causing leakages from liposomes. (Raqueel, 2007). In addition to this the anti-bacterial activity of aqueous leaf extract against both gram-positive and gram-negative bacteria is indication of the presence of broad spectrum antibiotic compounds. This is of immense advantage in fighting menace of antibiotic resistance that is prevalent these days.

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