Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 02, pp.65978-65981, February, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

QUALITATIVE PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTI-BACTERIAL POTENTIAL OF ETHANOLIC LEAF EXTRACT OF CLERODENDRUM SERRATUM LINN. AGAINST SOME PATHOGENIC BACTERIA

[1,*]Malik Tafazul Rashid., [2]Yadav A.S. and [2]Shabir A. Lone

[1]Ex-Post Doctoral Researcher, Louisiana State University (LSU), USA
[2]Molecular Biology and Seed Technology Laboratory, Govt. MotilalVigyan Mahavidyalaya (MVM), Bhopal-08

ARTICLE INFO

Article History:
Received 21st November, 2017
Received in revised form
18th December, 2017
Accepted 12th January, 2018
Published online 28th February, 2018

Key words:
Clerodendrum Serratum Linn,
Staphylococcus Hominis,
Pseudomonas putida,
Proteus Vulgaris,
Bacillus Subtilis and Escherichia Coli.

ABSTRACT

The present study was designed to carryout the prelimnary photochemical screening and to assess the anti-bacterial potential of ethanolic leaf extract of *Clerodendrum searratum* LINN. Phytochemical investigation revealed the presence of alkaloids, phenolics, carbohydrates, proteins, carbonate and glycoside. In-vitro anti-bacterial activity was evaluated using disc diffusion method. The microorganisms used for anti-bacterial activity were *staphylococcus hominis* ATCC27844, *Pseudomonas putida* ATCC2021, *Proteus vulgaris* ATCC13315, *Bacillus subtilis* ATCC2063 and *Escherichia coli* ATCC2065. The most susceptible gram negative bacteria found were *Pseudomonas putida* ATCC2021 and *Escherichia coli* ATCC2065 with zones of inhibition ranging from 10±0.47mm to 23±0.22mm whereas the most susceptible gram negative bacteria found were *staphylococcus hominis* ATCC27844 followed by *Proteus vulgaris* ATCC13315 and *Bacillus subtilis* ATCC2063 with zones of inhibition ranging from10±0.23 mm to 20±0.23 mm. It was observed that with an increase in the concentration of the extract, there was an increase in the zone of inhibition and thus activity was found to be concentration dependent. Therefore, it was concluded from the results that the ethanolic leaf extract of *Clerodendrum searratum* LINN. Possess potent anti-bacterial activity and hence can be used for the treatment of various ailments caused by test organisms.

Citation: Malik Tafazul Rashid., Yadav A.S. and Shabir A. Lone, 2018. "Qualitative phytochemical investigation and evaluation of anti-bacterial potential of ethanolic leaf extract of clerodendrum serratum linn. against some pathogenic bacteria", *International Journal of Current Research*, 10, (02), 65978-65981.

INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread multiple drug resistance due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Akereleal *et al*., 1988). Moreover, antimicrobial drugs are sometimes associated with adverse effects on the host including hypersensitivity, immune-supression and allergic reactions (Zhang *et al*., 1998). Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. The success story of chemotherapy therefore lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. Medicinal plants, which form the backbone of traditional medicine, from the last couple of decades are the subject of very intense pharmacological studies Cowan *et al*. (1999); Harborne *et al*. (1998). In this connection, higher plants continue to be a rich source of therapeutic agents since they produce hundreds to thousands of diverse chemical compounds as secondary metabolites with different biological activities (Baur *et al*., 1966). The compounds produced by plants are active against plant and human pathogenic microorganisms. The remarkable contribution of plants to the drug industry was possible, because of the large number of phytochemical and biological studies carried out all over the world. Herbal remedies used in the folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. The most promising plant extract was selected for further phytochemical and pharmacological activities. This is in pursuance of the efforts to search for drugs from plants and the

******Corresponding author:* **Malik Tafazul Rashid,**
Ex-Post Doctoral Researcher, Louisiana State University (LSU), USA.

verification of the scientific basis of someknown practices in traditional medicine.

***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.

MATERIALS 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-bacterialassay

The anti-bacterialactivity of ethanolic 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 ethanolic 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

The etanobotanical efficacy of ethanolic leaf extract of *Clerodendrum serratum* Linn. against pathogenic bacterial strains showed varied level of inhibition.Among treatments,maximum in-vitro inhibition of tested bacteria was observed at a concentration of 100mg/ml of ethanolic leaf extract with zones of inhibition ranging from 17±0.40 mm to 23±0.22 mm.

Table 1. Results of phytochemical analysis of *leaves of Clerodendrum serratum* Linn.

Test	Results
Carbohydrates	+ve
Alkaloid	+ve
glycoside	+ve
Phenolics	+ve
Proteins	+ve
Flavonoids	+ve
Carbonate	+ve
Saponin	-ve
Steroid	-ve
Starch	-ve

+ve = Presence of constituents; -ve = Absence of constituents.

At all four concentrations i.e., 25, 50, 75 and 100 mg/ml of ethanolic leaf extract, *Pseudomonas putida* ATCC2021 showed maximum inhibition with zones 14±0.21 mm,18±0.21 mm, 20±0.31 mm and 23±0.22 mm which was followed by *Staphylococcus hominis* ATCC27844 with inhibition zones of

Table 2. Anti-bacterial activity of ethanolic leaf extract of *Clerodendrum searratum* Linn.

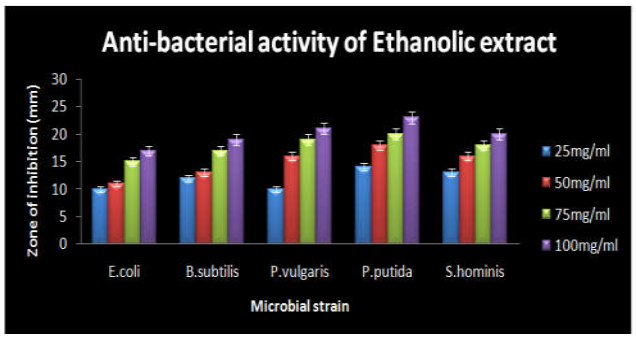
Microbial train	Concentration of Ethanolic Extract				Standard drug
	25mg/ml	50mg/ml	75mg/ml	100mg/ml	10 µg/ml
E.coli	10±0.47	11±0.40	15±0.64	17±0.40	18±0.47
B.subtilis	12±0.28	13±0.40	17±0.28	19±0.25	30±0.28
P.vulgaris	10±0.23	16±0.33	19±0.30	21±0.24	25±0.23
P.putida	14±0.21	18±0.23	20±0.31	23±0.22	16±0.21
S.hominis	13±0.20	16±0.22	18±0.21	20±0.23	24±0.20

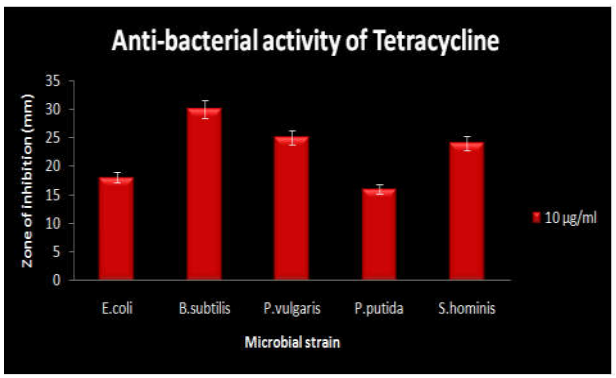
Values are mean inhibition zone(mm)±S.D of three replicates

13±0.20 mm, 16±0.22 mm, 18±0.21 mm and 20±0.23 mm. This is followed by *Proteus vulgaris* ATCC13315 with zones of inhibition of 10±0.23 mm, 16±0.33 mm, 19±0.30 mm, 21±0.24 mm and *Bacillus subtilis* ATCC2063 with inhibition zones of 12±.28 mm,13±0.40 mm, 17±0.28 mm and 19±0.25 mm. This is lastly followed by *Escherichia coli* ATCC2065 with inhibition zones of 10±0.47 mm, 11±0.40 mm, 15±o.64 mm and 17±o.40 mm respectively. Incase of pathogenic bacteria *Escherichia coli* ATCC2065 maximum inhibition of 17±0.40 mm was obtained at a concentration of 100 mg/ml of ethanolic leaf extract and the minimum inhibition of 10±0.47 mm was obtained at a concentration of 25 mg/ml of ethanolic leaf extract. Similarly for bacteria *Bacillus subtilis* ATCC2063, *Proteus vulgaris* ATCC13315, *Staphylococcus hominis* ATCC27844 and *Pseudomonas putida* ATCC2021, the maximum inhibition was observed at a concentrat.ion of 100mg/ml of ethanolic extract with inhibition zones of 19±0.25 mm, 21±0.24 mm, 20±0.23 mm and 23±0.22 mm and minimum inhibition was recorded at a concentration of 25 mg/ml with inhibition zones of 12±0.28 mm, 10±0.23 mm, 13±0.20 mm and 14±0.21 mm respectively.

Table 3. Minimum inhibitory concentration of aqueous leaf extract of Clerodendrum Serratum LINN

Microbial Strain	MIC (mg/ml)
Escherichia coli	6.74
Bacillus subtilis	7.56
Proteus vulgaris	7.65
Pseudomonas putida	8.96
Staphylococcus hominis	8.35





Based on our results, it was found that ethanolic extract showed significant activity against all tested bacterial strains. The zone of inhibition was found to increase with an increase in the concentration of the extract and thus exhibiting concentration dependent activity. Therefore, it was concluded that plant extracts have great potential as anti-bacterialagents and can be used for the treatment of infectious diseases caused by pathogenic bacterial strains.

DISCUSSION

The ethanolic extract of *Clerodendru Serratum* Linn. was subjected to a prelimnary screening for anti-bacterialactivity against five pathogenic bacterial strains. It was clear from the present results that the ethanolic extract exhibited pronounced anti-bacterialactivity against all tested bacteria. This tends to show that the active ingredients of the plant parts are better extracted with methanol than oter solvents. It may be due to high polarity of these solvents which naturally has the ability of extracting of high quantity of phytochemicals (Phillipson *et al*., 1987). Hence, it is understood that the ethanolic extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest anti-bacterial activity**.** On prelimnary phytochemical investigation, the ethanolic leaf extract of *Clerodendrum Serratum* Linn. revealed the presence of diverse secondary metabolites which includes alkaloids, phenolics, carbonate, glycoside, proteins and carbohydrates. Nearly all of the identified compounds from plants active against microorganisms are aromatic or saturated organic compounds obtained through initial methanol extraction. The bioactive compounds are known to act by different mechanism and excert anti-bacterialaction. The alkaloids, heterocyclic nitrogen containg compounds, are potent anti-bacterial agents whose activity is attributed to their ability to intercalate with bacterial DNA causing death of the microorganism (Bose *et al*., 1958). Among phenolics, tannins which are polymeric substances having well known anti-bacterialactivity and their mode of action may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins.Also phenolic substances called ciumarins are known to be highly anti-bacterialand their action is attributed to their ablity to intercalate with bacterial DNA Hoult *et al*., 1996; Minh Hien Ha *et al*., 2009 Parekh *et al*., 2007). Moreover, carbonate was also detected in the ethanolic leaf extract, is a reactive anion that forms insoluble divalent metal ion complexes and resulted in imhibition of enzyme activity. It was evident from the results that the bacteria Escherichia coli ATCC2065 was found to be least susceptible to ethanolic extract due to the presence of cell wall which might have halted the entry of phytoconstituents into the cell and resulted in less sensitivity to the extract (Abdulla *et al*., 2009). Furthermore, Pseudomonas putida ATCC2021 was found to be highly susceptible to ethanolic extract that may be related to its cellwall which might have allowed easy penetration of phytoconstituents and thus resulted in

appreciable anti-bacterialactivity. The other bacterial strains have shown moderate activity when compared to positive control tetracycline. Based on our results, it can be concluded that plant extracts have a great potential anti-bacterialcompounds against microorganisms that can be used in treatment of infectious diseases caused by a range of miocroorganisms. Therefore, the traditional medicinal methods especially the use of medicinal plants, still play a vital role to provide primary health care.In this manner, plants continue to be a rich source of therapeutic agents. It is anticipated that phytochemicals with adequate anti-bacterialefficiency will be used for the treatment of bacterial and fungal infections. Therefore, the need of the present hour is to screen a number of plants that were traditionally used and also to evaluate their probable phytoconstituents having potent anti-bacterialactivity.

Conclusion

The results of our study clearly indicate that the plants are valuable sources of potent anti-bacterialcompounds that have worthwhile efficiency in combating diverse ailments caused by pathogenic bacteria. Therefore, screening of various natural organic compounds and identification of active agents is the need of the hour because prediction of lead molecules and drug like properties at the inception of drug discovery will pay off later in drug development. It is imperative that research strategies must be oriented towards discovery and development of anti-bacterialagents urgently requirted in the future.

Acknowledgement

We are sincerely thankful to Molecular Biology and Seed Technology Laboratory and centre for microbiology and biotechnology for providing necessary facilities.

REFRENCES

Abdulla, S.S., Kumar, M., Marimuthu, R., DurgaLakshmi, R., Saraswathi, K., 2009. Screening ofphytoconstituents and anti-bacterialactivity of Gynandradropsis gynandra L.Briq. *Plant Archives*; 2: 591-594.

Akerele, O., 1988. Medicinal plants and primary health care: an agenda for action. *Fitoterapia,* 59: 355–363.

Baur, A.W., Kirby, W.M., Sherris, J.C., Turk, M., 1966. Antibiotic susceptibility testing by a standard single disk method. *Am J Clin path*; 45:493-49

Bose, P.K., 1958. On some biochemical properties of natural coumarins. *J. Indian Chem. Soc;* 58:367–375

Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev*., 12(4): 564–582.

Harborne, J.B., 1998. Phytochemical Methods, 3rd ed. London: *Chapman Hall*, 1998. 1–302.

Hoult, J.R., Paya, S., 1996. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential**.** *Gen. Pharmacol*; 27:713–722.

Marjorie, M.C., 1999. Plant products as anti-ibacterialagents**.** *Clin Microbiol Rev*., 12(4): 564-582.

Minh, H.H., Van Thi Nguyen, V.T., Nguyan, Q.K.Q., Emily, L.C., Cheah, Paul, W.S., Heng, 2009. Anti-bacterialactivity of Calophyllum inophyllum crude extracts obtained by pressurized liquid extraction. *Asian Journal of Traditional Medicines*, 4(4): 141-146

Parekh, J., Chandra, S., 2007. Anti-bacterialand phytochemicalstudies on twelve species of Indian medicinalplants. *Af J Biomed Res*., 31: 53-58

Phillipson, J.D., Neill, M.J., 1987. New leads to the treatment of protozoal infections based on natural product molecules. *Acta Pharm. Nord*; 1:131–144.

Zhang, X., 1998. Regulatory Situation of Herbal Medicines. *A Worldwide Review*. WHO: Geneva, Switzerland, 1–5.

****