

Phytochemical Analysis and Antibacterial Activity of *Clerodendrum philippinum* Schauer

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ABSTRACT

Natural drugs always play vital role in the modern medicine due to less dangerous than artificial drugs and provide deep restorative benefits. In some cases, it is generally used to cure some ailments which may not be treated by conventional medicine and that may be due to availability of biological active compounds. In this regard, *Clerodendrum philippinum* plant was evaluated for its secondary metabolites and antibacterial activity. Phytochemical screening revealed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins, phenolics and glycosides as major components. The ethanolic extracts of leaves were subjected for high performance thin layer chromatography (HTPLC) towards develop the chemical fingerprints. The profile confirms the presence of alkaloids, flavonoids, glycosides and terpenoids in various degrees of leaf extracts. The antibacterial activity was determined against pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Bacillus subtilis*, *Corynebacterium kroppenstedtii* and *Vibrio cholerae* by disc diffusion method. *C. philippinum* showed potent antibacterial activity against both Gram positive and Gram negative bacteria which provide evidence to support traditional medicinal uses of this plant or to develop new pharmaceutical research activities.

Keywords: Antibacterial, *Clerodendrum philippinum*, ethanolic extracts, HPTLC, phytochemical.

INTRODUCTION

Nature has created the plant kingdom, which is known as the source of natural medicine and able to remediate about all ailment of mankind. For this, the plants have been added as a vital component of the healthcare system and been inherited from the commencement of human civilization¹. In Indian system of medicine namely Ayurveda, Unani and Siddha (apart from Homeopathy and Electropathy), the medicinal plants also act as an alternative source for treating several ailments because of their usage was multifarious. During the last decades the exploration of traditional medicine and its potent properties has been expanded globally. Moreover, the plants are boons and bliss for the mankind as they are the most potential sources of raw materials used for manufacture of drugs. Primarily the benefits of use of plants are safer than the synthetic alternatives, offering profound healing aids and more affordable². Scientific interest in the medicinal plants has burgeoned in recent time due to the enhanced efficiency of new plant derived drugs and rising concern about the side effect of modern medicines³.

Many plants in pharmacopoeia are reported that they are loaded with various metabolites which are used as natural medicines to treat various diseases including bacterial infections and are regularly used in various system of Indian medicine because of their minimal/ no side effects and cost effective⁴. According to World Health Organization (WHO) about 80 % of populations in developing countries rely on traditional medicine for their primary health care needs. The use of plants for prevention and treatment of various health ailments has been in practice from time immemorial and it was estimated that about 25 % of drugs prescribed are derived from plants. However, WHO's essential medicine list described about 252 drugs, out of which 11 % is exclusively of plant sources^{5,6}. Phytochemicals are naturally occurring compounds mainly found in all parts of plant like leaves, stem bark, fruits and roots which could be utilized as formulations of drugs. They are also known as secondary metabolites in the form of active compounds either as alkaloids, steroids, flavonoids, terpenoids, glycoside, saponia, tannins, phenolic compounds etc. Plant secondary metabolites are mostly responsible for antimicrobial activity due to presence of phenolics or polyphenols

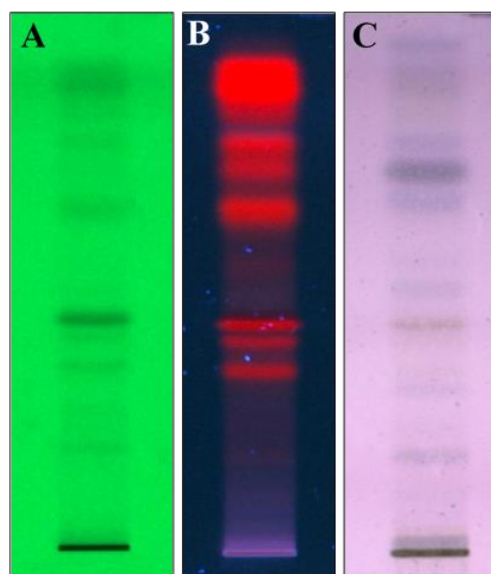


Figure 1: High performance thin layer chromatographic fingerprinting profile of ethanolic leaf extracts of *Clerodendrum philippinum*. A: Under UV 254 nm; B: Under UV 366 nm; C: Under visible light 550 nm.

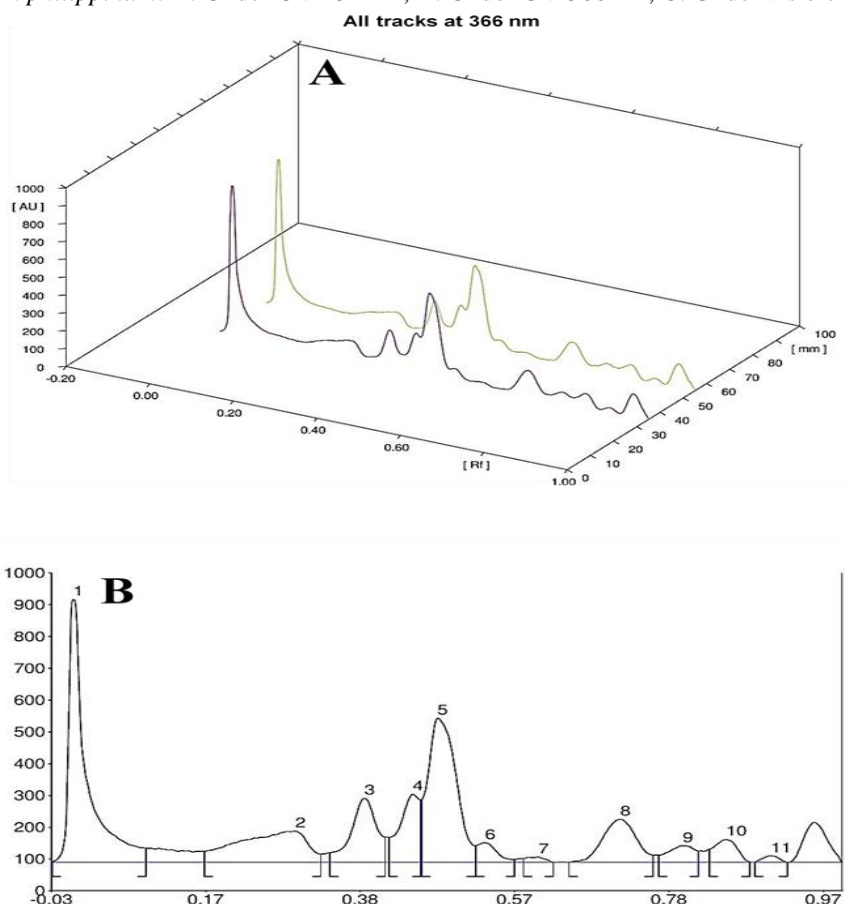


Figure 2: HPTLC chromatogram (A, B) of ethanolic extract at 366 nm, showing different peaks (bands) of phytoconstituents in *Clerodendrum philippinum*.

(flavonoids, quinones, tannins, coumarins), terpenoids, alkaloids, lectins and polypeptides^{6,7}. The main mechanisms that underlie antimicrobial action of plant-derived compounds are by disrupting microbial membranes (carvacrol, thymol, eugenol, etc.) or impairing cellular metabolism (cinnamaldehyde) or controlling

biofilm formation or inhibition of bacterial capsule production (salicylic acid and its derivatives)⁷.

Clerodendrum philippinum is an important medicinal plant that belongs to the family Verbenaceae. It is globally known as 'Chinese glory bower' and 'brajamalii' in the state of Odisha, India. The plant is mainly distributed in

Table 1: Preliminary phytochemical screening from different leaf extracts of *Clerodendrum philippinum*.

Extracts	Alkaloids	Terpenoids	Flavonoids	Saponins	Tannins	Steroids	Phenolics	Glycosides
Aqueous	-	-	+	+	+	-	+	++
Methanol	+	+	+	-	+	-	+	+
Ethanol	+	++	++	++	++	+	+++	+
Chloroform	+	-	-	-	-	-	+	+
n-Hexane	+	++	-	-	-	+	-	+

+++ : Strongest response; ++ : Strong response; + : Positive response; - : Negative response

Table 2: Mobile phase and spray reagent used for various secondary metabolites by using HPTLC for ethanolic leaf extracts of *Clerodendrum philippinum*.

Compound name	Mobile phase	Spray reagent
Alkaloids	Ethyl acetate-methanol-water	Dragendorff's reagent followed by ethanolic sulphuric acid
Flavonoids	Toluene-ethyl acetate-acetic acid	Ethanolic aluminium chloride
Glycosides	Ethyl acetate-ethanol-water	Anisaldehyde sulphuric acid
Saponins	Chloroform-glacial acetic acid-methanol-water	Anisaldehyde sulphuric acid
Terpenoids	n-Hexane-ethyl acetate	Anisaldehyde sulphuric acid

Sourthen part of Asia and in India, abundantly available in various states like Karnataka, Kerala, Tamil Nadu and including Odisha. It is a semi-woody shrub which grows as ornamental and spreads as vegetative⁸. It is generally used to treat colic pain and exhibited antifungal activity. Root and leaf extracts have been found best fit against rheumatism and asthma. In some cases, it is also used for curing of jaundice, syphilis and typhoid⁹. The leaf juice is also used externally for scabies, cuts and burns. It was reported that the leaf juice when mixed with *Ocimum sanctum*, reduces sugar content^{8,10}. From the above existing information, it is evidenced that the plant possesses different biological active compounds. Therefore, in present study, an attempt is made to explore the phytochemical constituents and antimicrobial activity of *C. philippinum* from leaf extracts.

MATERIALS AND METHODS

Collection of plant material

Fresh and healthy leaves of *C. philippinum* were collected from the botanical garden of Post Graduate Department of Botany, Utkal University. The collected samples were identified and validated by taxonomist Dr. P. C. Panda, Principal Scientist, Regional Plant Research Centre, Bhubaneswar, Odisha. Then the voucher specimens of the plant species were deposited to the herbarium of Botany Department, Utkal University with voucher No. BOTU-10751.

Preparation of the leaf extract

The collected leaf samples were washed with running tap water to remove dust along with other solid particles and allowed to dry under shaded condition. Then dried samples were coarsely powdered using a mortar and pestle. The finely powdered samples were extracted with aqueous (distilled water), methanol, ethanol, chloroform and n-hexane in a Soxhlet apparatus for 12-14 h. Extracts were concentrated on a rotary evaporator and stored at -4 °C.

Screening of phytochemicals

Phytochemical screening of various organic leaf extracts were done according to the standard procedure by Harborne (1998)¹¹. The various organic extracts were

subjected for preliminary screening to find out the presence of active principles such as alkaloids (Wagner's test), terpenoid (Salkowski's test), flavonoids (NaOH test), saponin (foam test), tannins (Braymer's test), steroids (sulphuric acid test), phenol (ferric chloride test), glycosides (Keller-Kiliani's test) using standard procedures.

HPTLC analysis

HPTLC finger printing study was carried out by taking 100 mg of ethanolic extract of leaf samples which dissolved in 1 ml of HPTLC grade ethanol and centrifuged at 3000 rpm for 5 mins. These solutions were used as test solution for HPTLC analysis. A CAMAG (Muttens, Switzerland) HPTLC system, comprising a Linomat 5 automatic applicator with a 100 µl syringe, a twin trough plate development chamber, Camag TLC scanner 3, and WIN CATS software was used. Suitable volume of standard (2 µl) and sample solution (2 µl) were spotted in the form of bands having band width of 5 mm on precoated silica gel 60 F₂₅₄ HPTLC plate (Merck-India, Mumbai). Iodine vapour was applied for prederivatization by exposing the plate for 10 minutes. The prederivatized plate was developed vertically ascending in a twin through glass chamber (Camag, Switzerland) saturated with respective mobile phase for alkaloids, flavonoids, glycosides and terpenoids. The optimized chamber saturation time for the mobile phase was 20 mins at room temperature. The chromatographic run length was 90 mm from the bottom edge of the plate. Subsequent to the development, HPTLC plates were dried in an oven for 5 mins at 60 °C. Densitometric scanning was performed with a TLC scanner equipped with WIN CATS 1.4.2 software (Camag, Switzerland) in reflectance absorbance. The slit dimensions were 6mm × 0.45mm, scanning speed was 20 mms⁻¹, data resolution 100 µm/ step, optical filter (second order), and filter factor (Savitzky-golay 7). Plates were scanned at 254 nm which was selected experimentally on the basis of distinctive absorption spectra of the compounds between 200 and 400 nm. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV 254 nm,

Table 3: HPTLC Peak table with R_f values, height and area of alkaloids (A), flavonoids (B), glycosides (C) and terpenoids (D) from ethanolic leaf extracts of *Clerodendrum philippinum*.

Peaks	R _f values				Area (AU)			
	A	B	C	D	A	B	C	D
1	0.10	0.02	0.09	0.10	17490.6	660.7	13488.0	876.4
2	0.32	0.12	0.14	0.18	8057.8	1760.3	445.4	201.7
3	0.41	0.52	0.24	0.22	6073.7	478.1	1620.3	412.6
4	0.45	0.58	0.32	0.31	5028.2	563.0	900.8	1118.3
5	0.52	0.65	0.40	0.46	15073.1	1152.6	1580.9	2217.1
6	0.58	0.70	0.52	0.53	1456.4	1324.1	9550.8	2892.0
7	0.63	0.72	0.57	0.61	381.0	1768.2	842.1	13921.4
8	0.76	0.77	0.63	0.71	5345.5	1534.8	491.3	16238.1
9	0.81	0.82	0.75	0.78	1675.2	1321.0	1635.7	3998.3
10	0.88	0.91	0.87	0.80	1955.3	456.2	466.4	7632.6
11	0.93	0.97	0.93	0.88	402.5	791.2	603.3	5002.1

366 nm and visible light at 550. The peak numbers with its height, area, peak display and peak densitogram were identified.

Antibacterial activity

The bacterial strains [*Escherichia coli* (MTCC 1687), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 424), *Streptococcus pneumonia* (MTCC 655), *Bacillus subtilis* (MTCC 441)] obtained from Institute of Microbial Technology, Chandigarh and identified strains of *Corynebacterium kroppenstedtii* (SPAB2) and *Vibrio cholerae* (SPAB1) were used to evaluate the antibacterial activity.

The disc diffusion method was used to screen the antibacterial activity¹². *In vitro* bacterial activity was screened by using Nutrient Agar medium obtained from Himedia (Mumbai). The medium plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The different leaf extracts (aqueous, methanol, ethanol, chloroform and n-hexane) of *C. philippinum* were soaked on 6 mm sterile disc. The soaked disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The antibiotic, Ofloxacin (50 units/ disc) was used as a positive control for antibacterial activity.

Statistical analysis

All results (except HPTLC) are the mean of three independent experimental replicates (n = 6) and data is reported as mean ± standard error. Statistical analysis of the data were carried out using by analysis of variance (ANOVA) and tested for least significance differences (LSD) by Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Initially phytochemical analysis are done for detection different constituents such as alkaloids, terpenoid, flavonoids, saponin, tannins, steroids, phenol, glycosides from leaf extracts of *C. philippinum* which are responsible for pharmaceutical value. The study revealed that among

various extracts (aqueous, methanol, ethanol, chloroform and n-hexane), the ethanolic extract was rich in tested secondary metabolites followed by methanol, aqueous, n-hexane and chloroform which was tabulated in Table 1. However, different qualitative analysis indicates the presence of bioactive compounds. Plants are always served to treat different diseases of human kingdom which is due to presence of phytochemical compounds such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc.¹³. Therefore, preliminary screening is important to discovery of drugs and subsequent development. The presence of alkaloids show various biological activities like, anti-inflammatory, antimalarial, antimicrobial, cytotoxicity, antispasmodic and pharmacological effects¹⁴. Similarly, tannins have antibacterial, antitumor and antiviral activities¹⁵. According to Olaleye (2007), saponins are glycosides of both triterpene and steroids having hypotensive and cardiac depressant properties whereas steroids have great role as nutrition, herbal medicine and cosmetics.^[16] Plant derived phenolic compounds contribute towards scavenging free radical and act as primary antioxidants¹⁴. HPTLC profile of ethanolic extract was generated in different solvent systems of various polarities in order to determine the total number of phytochemicals (Table 2). After scanning and visualizing, the plates are exposed both UV light at 254 nm, 366 nm and visible light range of 550 nm after spraying with anisaldehyde sulphuric acid reagent) and best results were shown at 366 nm (Figure 2). HPTLC colored spots were visualized (clearly separated without any tailing and diffuseness) under the UV (254 and 366 nm) and visible light (550 nm) after sprayed with specific spraying reagents, thus indicating the presence of phytoconstituents (Figure 1). Further, Table 3 shows the presence of various alkaloids, flavonoids, glycosides and terpenoids with different retention factor (R_f) values and areas, which confirms the presence of respective compounds. Similar findings also observed in different plant species such as *Solena amplexicaulis*¹⁷, *Cassia fistula*¹⁸, *Evolvulus alsinoides*¹¹ and *Decalepis hamiltonii*²⁰ in order to search the different secondary elements. However, selection of solvent system towards extraction and plant parts used are different.

Table 4: *In vitro* antibacterial activities of different leaf extracts of *Clerodendrum philippinum*.

Extracts	Zone of inhibition (mm)						
	Microorganisms						
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. pneumoniae</i>	<i>B. subtilis</i>	<i>V. cholerae</i>	<i>C. kroppenstedtii</i>
Control (Ofloxacin)	10.9 ± 0.2 ^c	12.6 ± 0.1 ^{bc}	18.7 ± 0.2 ^{ab}	17.4 ± 0.3 ^{ab}	11.1 ± 0.3 ^{bc}	19.2 ± 0.3 ^{ab}	16.1 ± 0.6 ^b
Aqueous	12.3 ± 0.1 ^{bc}	9.8 ± 0.2 ^c	10.4 ± 0.2 ^c	6.2 ± 0.1 ^{de}	6.4 ± 0.1 ^{de}	5.1 ± 0.1 ^{de}	7.2 ± 0.0 ^{cd}
Methanol	12.9 ± 0.2 ^{bc}	8.4 ± 0.1 ^{cd}	11.7 ± 0.3 ^{bc}	13.2 ± 0.3 ^{bc}	10.7 ± 0.2 ^c	14.7 ± 0.3 ^b	9.4 ± 0.1 ^c
Ethanol	14.8 ± 0.3 ^b	9.9 ± 0.2 ^c	25.1 ± 0.5 ^a	15.2 ± 0.5 ^b	8.0 ± 0.4 ^{cd}	23.0 ± 0.5 ^a	19.8 ± .3 ^{ab}
Chloroform	4.3 ± 0.0 ^d	8.4 ± 0.1 ^{cd}	–	6.7 ± 0.1 ^{de}	–	4.3 ± 0.0 ^d	–
n-Hexane	6.2 ± 0.1 ^{de}	–	–	2.4 ± 0.1 ^e	–	3.9 ± 0.1 ^d	–

–: No zone of inhibition.

The data in the table represent mean ± SE of replicates (n = 6). Values in the table carrying different letters are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

The antibacterial activity of different leaf extracts (aqueous, methanol, ethanol, chloroform and n-Hexane) of *C. philippinum* were studied against different pathogenic bacterial strains, out of which, four strains are Gram positive (*S. aureus*, *S. pneumoniae*, *B. subtilis*, *C. kroppenstedtii*) and three are Gram negative (*E. coli*, *P. aeruginosa*, *V. cholerae*). The results are presented in Table 4 by assessing in terms of zone of inhibition of bacterial growth and compared with standard (Ofloxacin). Maximum zone of inhibitions was observed in ethanolic extract against *P. aeruginosa* (25.1 ± 0.5) and *V. cholerae* (23.0 ± 0.5), which was more significant than other tested extracts. The growth inhibition zone measured ranged from 2.4 - 25.1 mm for all the sensitive bacteria however, leaf extracts from chloroform and n-hexane was found less effective and/ no effective (Table 4). The above study provides significant information about broad range of antimicrobial activity and indicates that *C. philippinum* is an alternative source of antibacterial agents against *P. aeruginosa*, *V. cholerae*, *E. coli* and *C. kroppenstedtii*. Previously reported findings suggested the novelty of plant extract against few bacteria however, no clear information are there regarding impact of different solvent extract on antibacterial activity.^[21] Moreover indications are there, that the antimicrobial activities also vary with the solvents used. This tends to show that active ingredients of the leaf are better extracted with ethanol. Ethanol found to be more effective solvents in extracting phytochemicals from different plant materials²²⁻²⁵. The present finding determines the importance of solvents used in preparation of drugs, which could be acceptable as development of new drugs. Further, the leaf extract of *C. philippinum* was active against all the tested bacteria are also an indication of potent antibiotic substances which can be used against drug resistant microorganisms.

CONCLUSION

The medicinal plants serve as important healing agents which contain natural substances essential to promote health. They are also highly valuable raw materials for developing of numerous traditional and modern medicines. The present study revealed that ethanolic leaf extract of *C. philippinum* was rich in phytochemical constituents

(alkaloids, flavonoids, glycosides and terpenoids) and showed presence of antimicrobial properties against some pathogenic strains of bacteria. The leaf samples indicates as it riches with phenolics, flavonoid, tannin and saponins compounds, have been shown to possess antimicrobial activities. Present findings also justify that uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. The results indicate that the plant possess a significant source of natural drug compounds with health protective potential and contains natural antibacterial compounds for human health and disease prevention. Hence, isolation of the bioactive components through high performance liquid chromatography would be of interest for further studies.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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