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# Phytochemical Evaluation Of The Leaves Of Clerodendrum Paniculatum Linn.

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## Abstract

Plants produces various bioactive components such as phenols, terpenoids, tannins, glycosides, alkaloids, saponins etc that are components having potential medicinal properties. The plant *Clerodendrum paniculatum* (*Verbenaceae*) reported to possess various medicinal properties such as antioxidant, antimutagenic, anticancerous and as anti-inflammatory agent. In this present work the phytochemical evaluation of leaves were performed to evaluate the active principles responsible for exhibiting the medicinal potential of the related plant. The extracts were subjected to preliminary phytochemical evaluation for carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, steroids, saponins and phenolics compounds, quantitative estimations such as Chromatographic separations and spectral analysis. In chromatographic evaluation the fractions present in ethyl acetate extract were separated by using Thin Layer Chromatography and further fingerprinting were done using High Performance Thin Layer Chromatography. In Spectral analysis Ultra-Violet Spectroscopy and Infrared Spectroscopy were carried out for characterization of chemical compounds present in the plant *Clerodendrum paniculatum*. The result of this work will facilitate phytochemical standardization of this plant material for further studies.

**Keywords**

Phytochemical standardization, Preliminary phytochemical evaluation, Chromatographic evaluation, Spectral analysis.

## Introduction

The plant *Clerodendrum paniculatum* belonging to the family *Verbenaceae* is a bushy perennial herb or an

erect shrub with cordate-ovate, 3-5 lobed, 4-40 cm long leaves and orange red to scarlet colored, many flowered, axiliary and terminal cymes, found in Andaman and Nicobar islands and grown in rockeries. The plant is used as an abortifacient by tribals of Andaman and Nicobar islands[1]. And this species is spread widely also in Asia, Africa, America, and Australia. It is reported to have antioxidant, anti-inflammatory [2], bactericidal[3], anticarcinogenic and antimutagenic properties[4]. Phytochemical screening on the root and flowers of this plant confirmed the presence of glycosides, tannins, steroids, saponins, alkaloids, flavanoids and phenolic components are responsible for various medicinal properties[5]. However, its medicinal uses, pharmacognostical and physicochemical properties were not reported widely and its constituents are being investigated for other pharmacological properties and for potential in human medicine.

## Materials and Methods

### Plant Collection and Identification

The plant specimens for the proposed study were collected from Kanyakumari district and authenticated by Prof. P. Jayaraman, Ph.D, Director of Plant Anatomy Research Center, Chennai.

### Phytochemical Investigations

Phytochemical evaluation is used to determine the nature of Phytoconstituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. It can be done by confirmation with different chromatographic techniques like TLC and HPTLC. Therefore a complete investigation is required to characterize the Phytoconstituents qualitatively and quantitatively. The leaves of *Clerodendrum paniculatum* was subjected to various phytochemical studies for which the materials and methods are presented below:

### Extraction of Plant Material

The leaves of Clerodendrum *paniculatum* was collected and subjected to shade dry. The shade dried material was ground into coarse powder. The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Clerodendrum paniculatum* (90 gm) was first extracted with Petroleum ether (60-80°C) in Soxhlet apparatus and then with solvents of increasing polarity like chloroform, ethyl acetate, ethanol and water at (60 - 70ºC). They were then followed with distillation by using simple distillation method. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed Phytochemical and pharmacological screening.

### Preliminary Phytochemical Screening[6- 8]

The preliminary phytochemical screening for various phytoconstituents in the different extracts of *Clerodendrum paniculatum* were carried out and the results were recorded.

### Quantitative Estimation

### Chromatography [9-11]

Chromatography methods are important analytical tool in the separation, identification and estimation of components present in the plant.

### Thin Layer Chromatography

Thin Layer Chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase.

### Instrument Conditions

**Sample Used** : Ethyl acetate extract

**TLC Applicator** : Manual application

**Mobile Phase** : Toluene: Ethyl acetate (9:1)

**Stationary Phase** : TLC Silica Gel 60 F254 (Merck)

**Detecting Method:** UV Chamber

**High Performance Thin Layer Chromatography-Fingerprint Profile**

HPTLC is one of the versatile chromatographic method which helps in the identification of compounds and thereby authentication of purity of herbal drugs. The time required in this method for the demonstration of most of the characteristic constituents of a drug is very quick and short. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drug, thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of Phytoconstituents which is unique to each plant. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract.

**Instrument Conditions**

| | |
|---|---|
| **Sample used** | **:** Ethyl acetate extract |
| **Extract Instrument** | **:** CAMAG HPTLC |
| **HPTLC Applicator** | **:** CAMAG LINOMAT-5 |
| **HPTLC Scanner** | **:** CAMAG TLC SCANNER-4 |
| **Sample Dilution** | **:** 100mg of sample extracted with 10 ml of Ethyl acetate |
| **Volume of injection** | **:** 100 µL |
| **Mobile Phase** | **:** Toluene: Ethyl acetate (9:1) |
| **Lamp** | **:** Deuterium |
| **Stationary Phase** | **:** TLC silica gel 60 F254 (Merck) |
| **Equipment** | **:** A Camag HPTLC system equipped with a sample applicator Linomat-5, Twin trough plate development chamber, TLC Scanner-4. |

**Chromatographic Conditions**

The estimation has been done using the following chromatographic conditions. Chromatography was performed on a 10 × 10cm pre-activated HPTLC silicagel 60 F254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat-5 with nitrogen flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner-4.

**Spectroscopical Studies**

Spectroscopic methods have become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of the pharmaceutical and biological materials.

**UV Spectral Analysis [12, 13]**

Ultraviolet - Visible Spectroscopy is related to the spectroscopy of photons in the UV (200 to 400 nm) - Visible region (400 to 800 nm). The colour of the chemicals directly affects the absorption and the molecules present in coloured solution undergo electronic transitions in visible ranges of spectrum.

**Infra-Red Spectral Analysis [14]**

Infrared Spectroscopy offers the possibility to measure different types of functional groups of atomic bond vibration at different frequencies. Especially in organic chemistry the analysis of IR absorption spectra shows that what types of bonds are present in the sample. It is also important method for analyzing polymers and constitution like fillers, pigments and plasticizers. IR Spectroscopy is the study of absorbed or transmitted or reflected radiation of electromagnetic spectrum from wavelength.

**Results and Discussion**

**Phytochemical Investigations**

**Extraction of Plant Material**

The yield of different successive extracts of dried powdered leaves are given in Table 1.

**Table.1.** Successive extracts of leaves of Clerodendrum paniculatum

| S. No. | Extract | Yield(%w/w) |
|---|---|---|
| 1. | Petroleum ether | 2.31% |
| 2. | Chloroform | 2.80% |
| 3. | Ethyl acetate | 3.0% |
| 4. | Alcohol | 13.55% |
| 5. | Aqueous | 16.07% |

**Preliminary Phytochemical Screening**

The qualitative chemical examination showed the presence of alkaloids, carbohydrates, glycoside, anthraquinone glycoside, steroids, flavonoids, phenols and saponins.

**Table.2.** Phytochemical screening of the extracts of *Clerodendrum paniculatum*

| S. No. | Chemical Constituents | Petroleum ether extract | Chloroform Extract | Ethyl acetate extract | Ethanol extract | Aqueous Extract |
|---|---|---|---|---|---|---|
| 1. | Alkaloids | - | + | + | + | + |
| 2. | Carbohydrates | - | + | + | + | + |
| 3. | Glycosides | - | + | + | + | - |
| 4. | Anthraquinone Glycosides | - | - | - | - | - |
| 5. | Steroids | + | + | + | + | + |
| 6. | Flavonoids | | | + | + | + |
| 7. | Saponins | - | - | - | + | + |
| 8. | Phenolics | - | - | + | + | + |
| 9. | Proteins | - | - | - | - | - |
| 10. | Tannins | - | - | + | + | + |

**+ve - Present, -ve - Absent**

**Quantitative Estimation**

The plant *Clerodendrum paniculatum* was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality.

**Chromatography**

**Thin Layer Chromatography**

Thin Layer Chromatogrphy of the various extracts were performed and $R_f$ value was determined for phenolic compounds.

**Table No.3:** Thin Layer Chromatography for Phenolic compounds

| S.No. | Extract | Solvent System | No. of Spots | $R_f$ Values |
|---|---|---|---|---|
| 1. | Petroleum ether | Toluene: Ethyl acetate (9:1) | 4 | 0.43<br>0.57<br>0.76<br>0.95 |
| 2. | Chloroform | Toluene: Ethyl acetate (9:1) | 4 | 0.53<br>0.67<br>0.74<br>0.80 |
| 3. | Ethyl acetate | Toluene: Ethyl acetate (9:1) | 3 | 0.38<br>0.88<br>0.94 |
| 4. | Ethanol | Toluene: Ethyl acetate (9:1) | 3 | 0.43<br>0.64<br>0.71 |
| 5. | Water | Toluene: Ethyl acetate (9:1) | - | - |

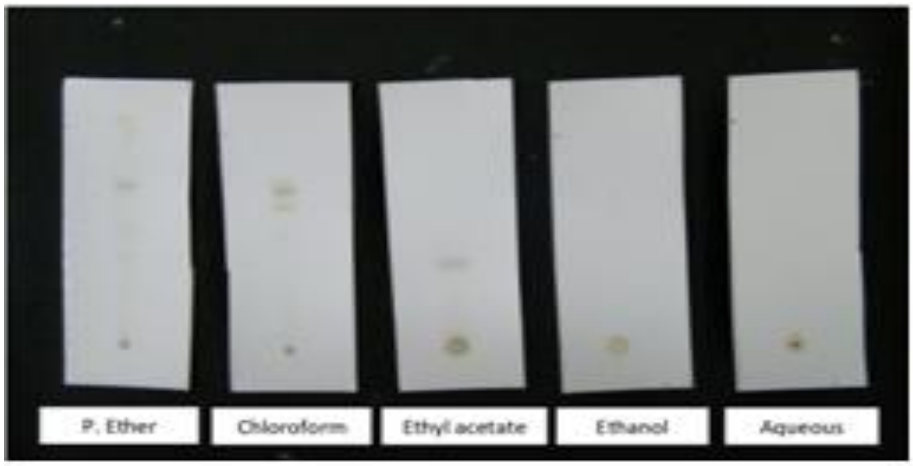


**Fig. 1.** TLC plate viewed in visible light

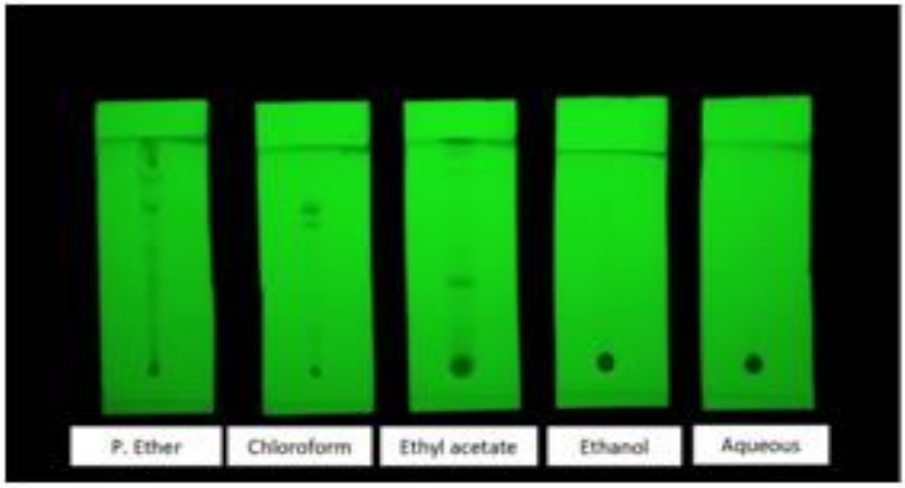


**Fig.2.** TLC plate viewed in UV light at 254 nm

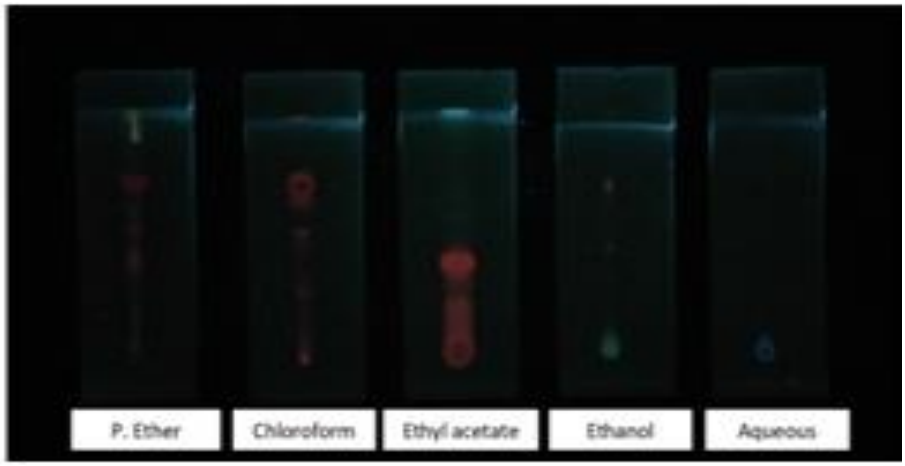


**Fig.3.** TLC plate viewed in UV light at 366 nm

**HPTLC- Fingerprint Profile**

HPTLC- fingerprinting was performed for the Ethyl acetate extract of *Clerodendrum paniculatum* leaf extract and the $R_f$ value was determined.

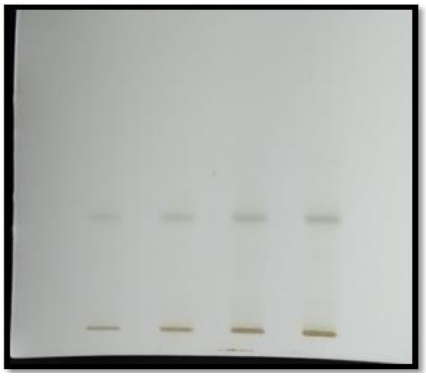

**Fig.4.** HPTLC Chromatogram viewed at visible light

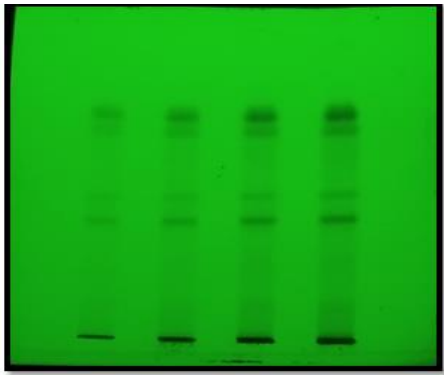

**Fig.5.** HPTLC Chromatogram viewed at 254nm

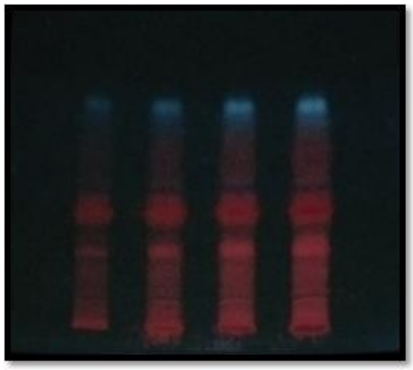

**Fig.6.** HPTLC Chromatogram viewed at 366 nm

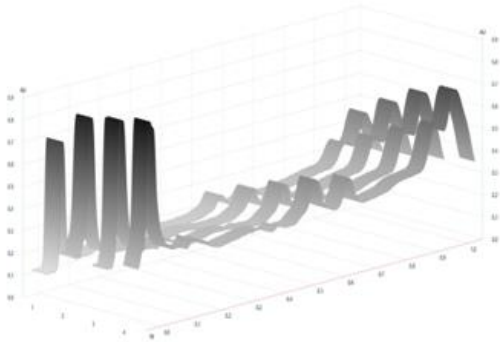

**Fig.7.** HPTLC all tracks at wavelength 254nm

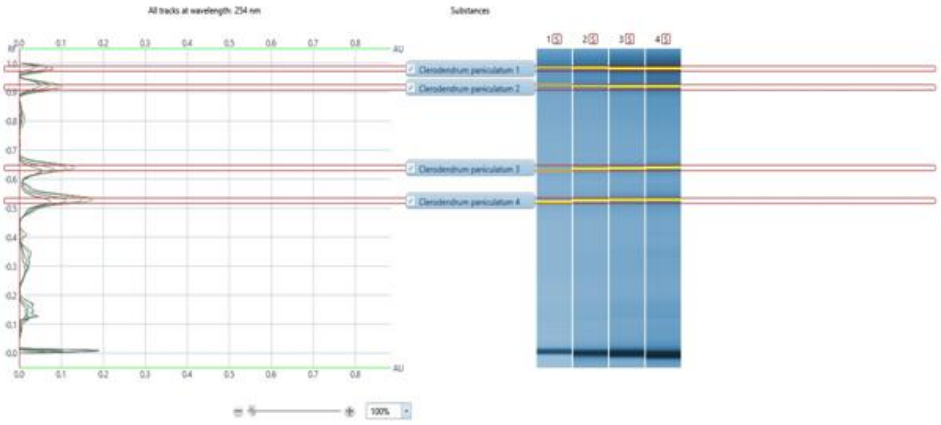


**Fig.8.** HPTLC of *Clerodendrum paniculatum* extract (All tracks at wavelength 254nm)

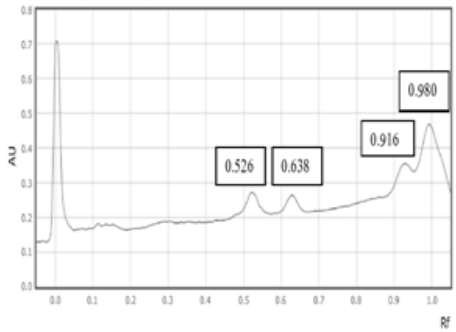


**Fig.9.** HPTLC Chromatogram at 254 nm (10µg/ml)

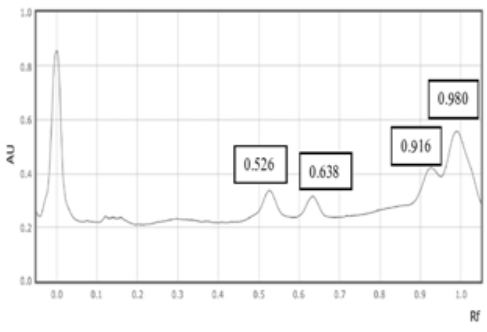


**Fig.10.** HPTLC Chromatogram at 254 nm (20µg/ml)

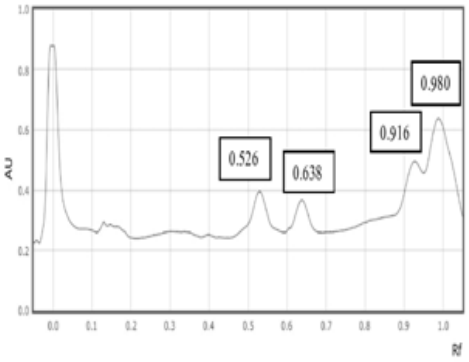


Fig.11. HPTLC Chromatogram at 254 nm (30µg/ml)

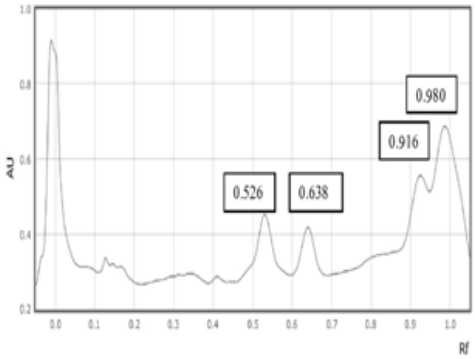


Fig.12. HPTLC Chromatogram at 254 nm (40µg/ml)

The Rf values obtained from the chromatogram was given in the following table No. 4

**Table No. 4:** Data showing the $R_f$ values of *Clerodendrum paniculatum* extract

| S.No. | Peak No. | λ | $R_f$ value |
|---|---|---|---|
| 1. | Peak 1 | 254nm | 0.980 |
| 2. | Peak 2 | 254nm | 0.916 |
| 3. | Peak 3 | 254nm | 0.638 |
| 4. | Peak 4 | 254nm | 0.526 |

**Spectroscopical Studies**

**UV Spectral Analysis**

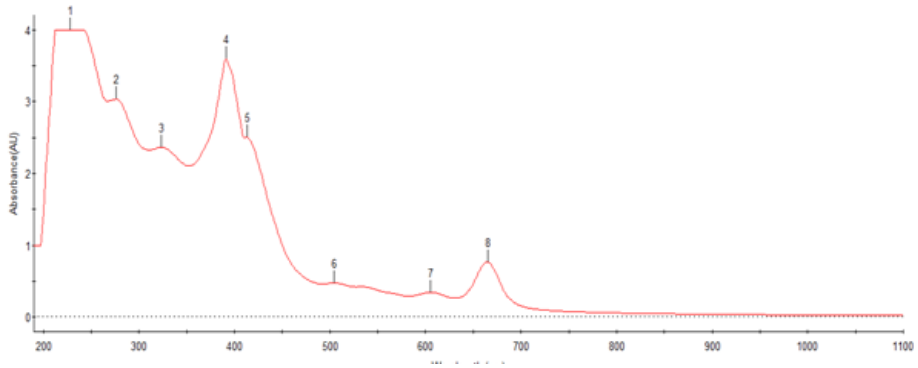


**Fig 13.** UV Spectrum of Ethyl acetate extract of *Clerodendrum paniculatum* Linn.

**Table No. 5.** Maximum Absorbance of Ethyl acetate extract of *Clerodendrum paniculatum* Linn. At different Wavelengths

| S. No. | Wavelength (nm) | Maximum Absorbance Maxima |
|---|---|---|
| 1. | 227.95 | 4.0000 |
| 2. | 276.15 | 3.0412 |
| 3. | 323.25 | 2.3646 |
| 4. | 391.30 | 3.5940 |
| 5. | 412.45 | 2.5043 |
| 6. | 504.30 | 0.4770 |
| 7. | 605.00 | 0.3479 |
| 8. | 664.50 | 0.7688 |

The UV spectra of the ethyl acetate extract of *Clerodendrum paniculatum* showed characteristic absorbances at 227 and 323 nm which are comparable with the standards

Gallic acid and Vannilic acid absorbances (227.95 nm and 320 nm)

**Infra-Red Spectral Analysis**

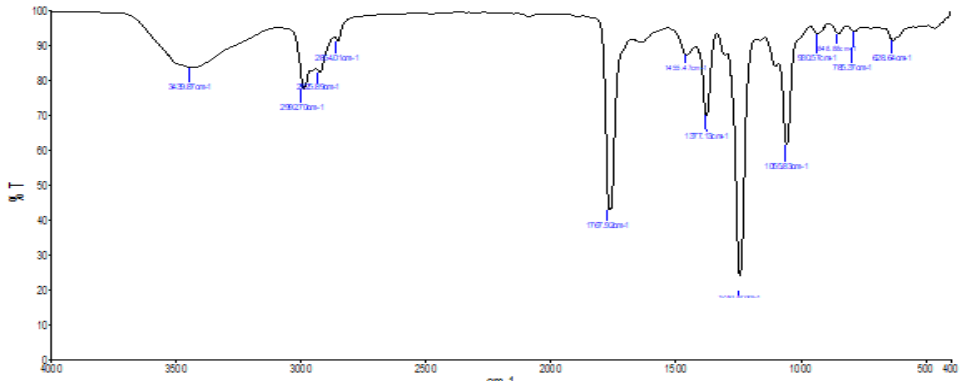

**Fig. 14.** IR Spectrum of Ethyl acetate extract of *Clerodendrum paniculatum* Linn.

**Table No.6.** FTIR interpretation of compounds of Ethylacetate of *Clerodendrum paniculatum* Linn.

| S.No. | Functional Groups | Peak values ($cm^{-1}$) |
|---|---|---|
| 1. | $C_{15}H_{12}O_7$ | 3439.87 $cm^{-1}$ |
| 2. | $C_6H_5(OH)_2$ | 1377 $cm^{-1}$ |
| 3. | -OH | 2864.01, 2975.89, 2992.7 $cm^{-1}$ |
| 4. | Chelated C-O | 1455.47 $cm^{-1}$ |

**Conclusion**

The phytochemical investigation on *Clerodendrum paniculatum* plant extracts shows that it contain biologically active secondary metabolites. The chromatographic separations for phenolic compounds revealed the presence of pharmacologically active phenolic compounds. The spectral studies helps in the confirmation of phenolic compounds present in the ethyl acetate extract of *Clerodendrum paniculatum.* The overall phytochemical investigation on *Clerodendrum paniculatum* reveals that the plant have significant medicinal properties and helpful to elucidate the pharmacological activity of *Clerodendrum paniculatum* in future research works.

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